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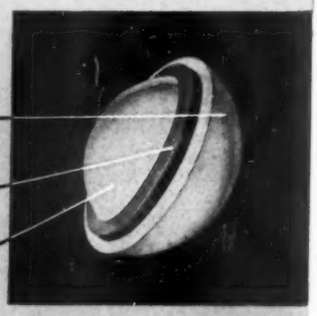

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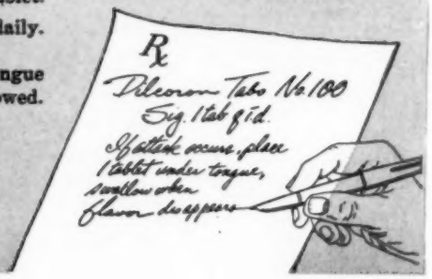

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The American Journal of Medicine

Vol. XXVI JANUARY 1959 No. 1

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Clinical Studies

Familial Hypercholesteremic Xanthomatosis: A Preliminary Report. I. Clinical, Electrocardiographic and Laboratory Considerations . . . J. L. GURAVICH 8

The data assembled in this extensive study of two kindreds afflicted with familial hypercholesteremic xanthomatosis again point up the pathogenetic relationships between hyperlipidemia and the clinical manifestations of xanthomatosis and atherosclerosis. Taking into account the factors of age and sex, there was a general although by no means absolute correlation between blood lipid levels and the development of xanthomas and coronary artery disease, overt or subclinical. Many additional points of interest relevant to the question of the role of serum cholesterol in atherogenesis are brought out.

Studies in Essential Hypercholesterolemia and Xanthomatosis. Relationships Between Age, Sex, Cholesterol Concentrations in Plasma Fractions, and Size of Tendinous Deposits . . . . . B. HOOD AND G. ANGERVALL 30

The results of this study conform with those of others indicating a continuum between essential hypercholesterolemia of modest degree without external or vascular xanthomatous deposits, at least early in life, and the more severe forms of the metabolic error characterized by marked hypercholesterolemia and extensive tissue deposits of cholesterol occurring relatively early in life. The distribution of cholesterol in Cohn fractions I + II + III and IV + V + VI also was investigated. The findings in general are in accord with previous reports but in this larger study show more spread than previously indicated.

Familial Hypercholesterolemia, Xanthomatosis and Coronary Heart Disease FREDERICK H. EPSTEIN, WALTER D. BLOCK, EUGENE A. HAND AND THOMAS FRANCIS, JR. 39

The authors have re-examined, after a lapse of ten years, a large kindred exhibiting a high familial incidence of hypercholesterolemia. They compare the current status with that recorded a decade ago. The association with manifest xanthomatosis was now more frequent, and the relationship to overt coronary heart disease, which was not clear in the earlier analysis, was now readily apparent. In the light of this retrospective experience the authors feel impelled to modify earlier interpreta-

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CLINICAL BRIEFS FROM MODERN PRACTICE

What differentiates "renal diabetes" (renal glycosuria) from diabetes mellitus?

Blood sugar levels. In renal glycosuria they are normal; in untreated diabetes, fasting blood sugars are usually 130 mg.% or over and postprandial levels 170 mg.%, or more.

Source: Joslin, E. P.; Root, H. F.; White, P., and Marble, A.: The Treatment of Diabetes Mellitus, ed. 9, Philadelphia, Lea & Febiger, 1952, pp. 701-702.

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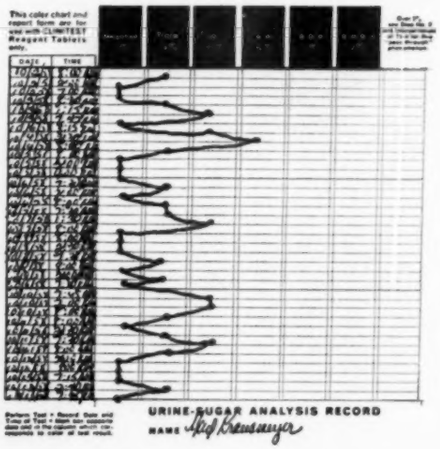
*GP *16*:121 (August) 1957.



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tions as to the genetic transference and clinical implications of the trait. They leave open the question whether xanthomatosis is the expression of a single gene or of a concurrence of two abnormal alleles.

Serum Lipids, Heredity and Environment. A Study of Adult Twins
RICHARD H. OSBORNE, DAVID ADLERSBERG, FRANCES V. DEGEORGE AND CHUNI WANG 54

In this ingeniously designed study the relative importance of hereditary and environmental factors affecting the serum cholesterol level (and hence presumably the predisposition to atherosclerosis) was investigated in monozygotic and dizygotic twin pairs. Monozygotic twins are genetically identical, and of course age and sex differences are eliminated, dizygotic twins are comparable to single born siblings in their genetic constitution, and may differ in sex. Environmental factors, including diet, could be dissociated to some extent according to whether the twins lived together or apart. While there are limitations to what can be achieved in such an analysis of an apparently healthy population, even in twins, the results indicate quite clearly that heredity as well as environment (diet) plays a role in the variability of the serum cholesterol level within the normal limits of variation. Genetic factors may not be equally significant in different environments, and vice versa.

The Mode of Inheritance in Essential Familial Hypercholesterolemia
KURT HIRSCHHORN AND CHARLES F. WILKINSON, JR. 60

There are three schools of thought as to how essential familial hypercholesterolemia is inherited: as a simple dominant gene variously expressed as xanthomatosis and/or hypercholesterolemia; as an incompletely dominant gene; as a recessive. The present study is an examination of these modes of transmission in the light of an analysis of one large and two small kindreds, and of the kindreds described by others. It is concluded that the available evidence supports inheritance of the trait by an incompletely dominant gene.

The Effect of Lipemia upon Coronary and Peripheral Arterial Circulation in Patients with Essential Hyperlipemia
PETER T. KUO, ARTHUR F. WHEREAT AND ORVILLE HORWITZ 68

The authors establish a quite convincing correlation between hyperlipemia and subjective and objective evidences of impaired coronary and peripheral circulation, in patients with essential hyperlipemia, by dietary manipulation to lower or increase their blood lipid levels. The findings would seem to settle in the affirmative the long disputed question whether very high serum triglyceride levels, by one mechanism or another, may increase arterial ischemia in patients with arteriosclerosis. The question has practical implications which are discussed.

The Influence of Anticoagulant Therapy on the Incidence of Thromboembolism, Hemorrhage and Cardiac Rupture in Acute Myocardial Infarction. Correlation of Clinical and Autopsy Data in 100 Cases
NICHOLAS E. CAPECI AND ROBERT L. LEVY 76

Although the use of anticoagulants in the management of acute myocardial infarction has been widely adopted, many physicians do not find the statistical evidence of benefit altogether convinc-

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▪ prompt, aggressive
antibiotic action
▪ a reliable defense against
monilial complications

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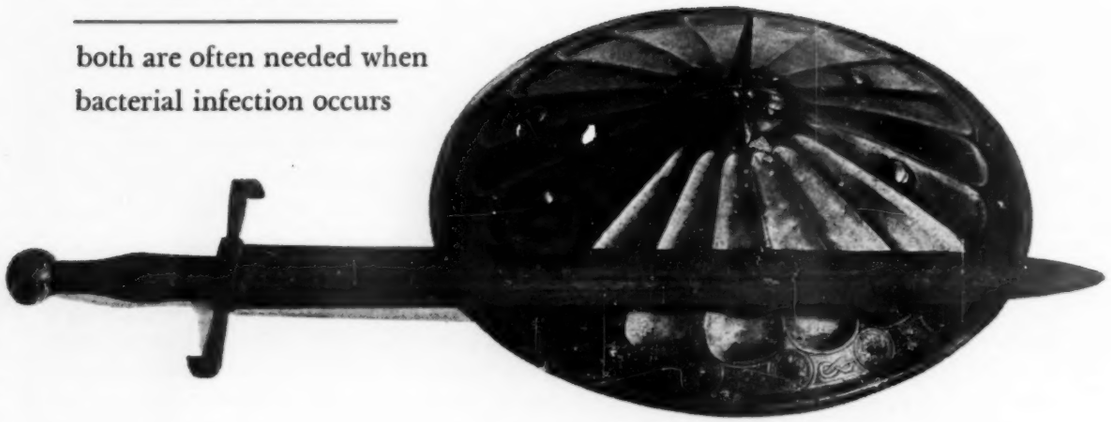
both are often needed when
bacterial infection occurs



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ing, and cite data to support their doubts. The present study is based on autopsy findings in 100 cases of myocardial infarction in which half the patients received anticoagulants. Such a group, as the authors take pains to point out, is a selected series which is of necessity biased. Nevertheless, postmortem examination permits a definitive evaluation of results which clinical appraisal may not, and this method of comparison therefore has a certain validity which deserves close attention. In this study the efficacy of anticoagulant therapy in prevention of thromboembolic complications could not be demonstrated. The incidence of myocardial rupture in a collective analysis of data appears indeed to be substantially higher in patients treated.

Review

Retinopathies Contrasted. Diagnostic and Prognostic Significance of the Optic Fundi in Accelerated Hypertension

ALBERT A. BRUST, WITH THE TECHNICAL ASSISTANCE OF LEONA WITHEROW 81

Dr. Brust has made a significant contribution to funduscopy in hypertension and other disorders affecting the eyegrounds, particularly in documenting progressive changes by means of reflex color photography, as illustrated profusely in this article. He begins with a brief description of the anatomy of the eyegrounds and of the characteristics of retinal hemorrhages in diabetes, hypertension, leukemia, subarachnoid bleeding and other disorders. He then discusses the distinctive features of retinal exudates, papilledema and the postpapilledema halo. This leads to a consideration of the progressive and regressive changes encountered in relation to hypertension, particularly as seen in malignant hypertension and chronic renal disease. The effect of modification of the blood pressure by antihypertensive drugs, as reflected in eyeground changes, is discussed, with emphasis on discordancies.

Seminar on Connective Tissue

Some Notes on the Composition and Metabolism of Connective Tissue

H. G. B. SLACK 113

Dr. Slack has brought together a great deal of current information concerning the nature of the components of connective tissue, in the process admirably sifting and integrating the available facts. The fibrous proteins (the collagens including reticulin, the elastins) and the non-fibrous proteins (including the plasma proteins, especially albumin) are first defined and described, then the polysaccharides such as hyaluronic acid, chondroitin, the chondroitin sulfates, keratosulfate, and heparitin sulfate. The author proceeds next to discuss the biosynthesis and metabolism of these constituents of the connective tissue, insofar as information is available through isotope labeling and other methods of investigation. The whole serves to provide a solid basis for consideration of the abnormalities of connective tissue which will be the subjects of papers to follow in this symposium.

Clinicopathologic Conference

Jaundice, Fluctuating Consciousness, Polyuria, Fever and Death 125

Clinicopathologic Conference (Washington University School of Medicine).

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*Fitzgerald, W.: Clinical Medicine, 5:1037, 1958.

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Chronic Histoplasmosis. Report of a Patient Successfully Treated with Amphotericin B
ROBERT M. GREENDYKE AND NOLAN L. KALTREIDER 135

A report of unusual interest, documenting a successful response to amphotericin B, given intravenously, in a patient with chronic histoplasmosis and wide systemic dissemination.

Idiopathic Insulin-Resistant Diabetes Mellitus. Report of a Case Associated with Insulin Allergy . . . ERNEST O. FRIEDLANDER AND M. DAVID BRYANT, JR. 139

An interesting example of a still obscure phenomenon, which is discussed in some detail.

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An informative report.

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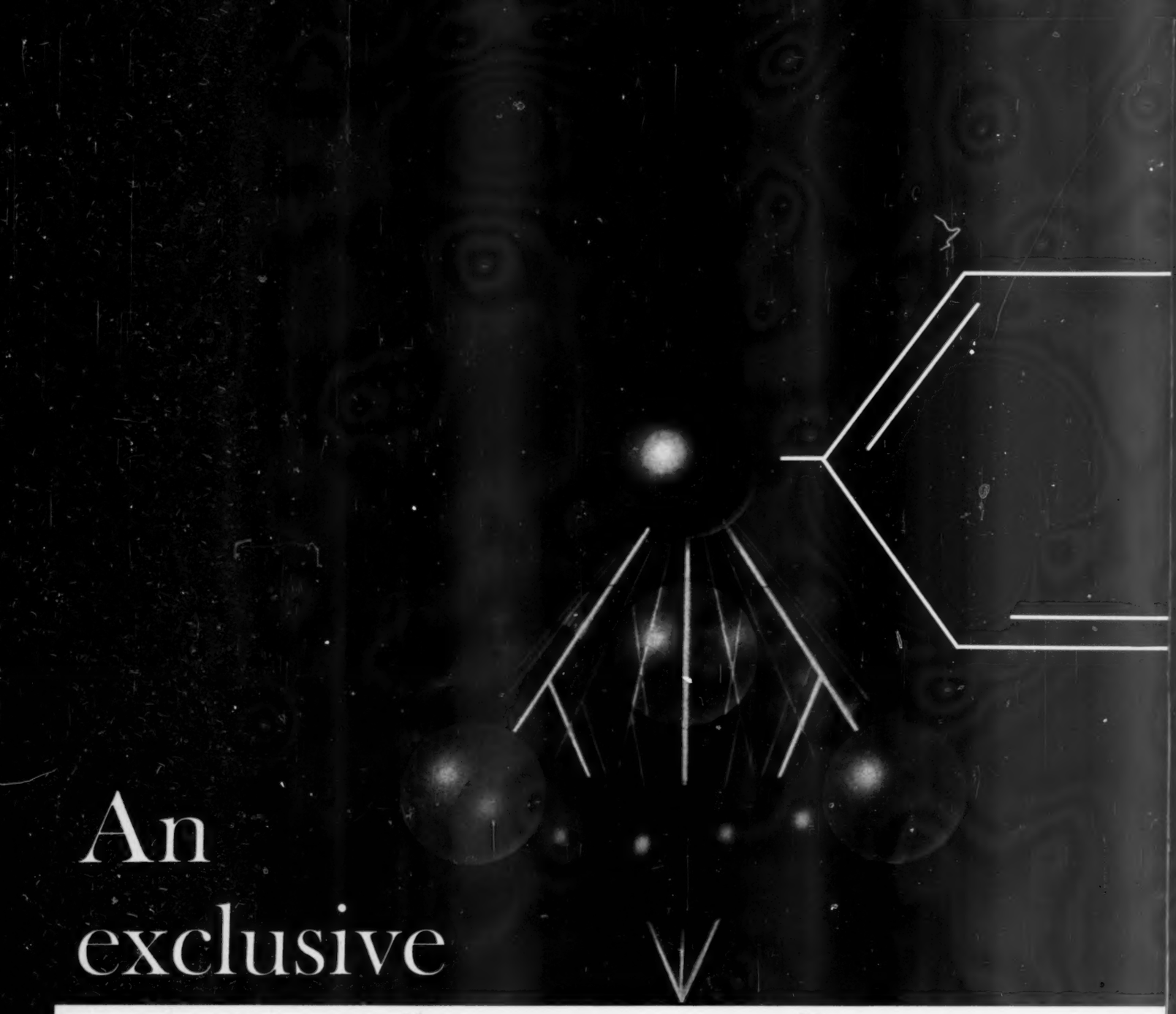

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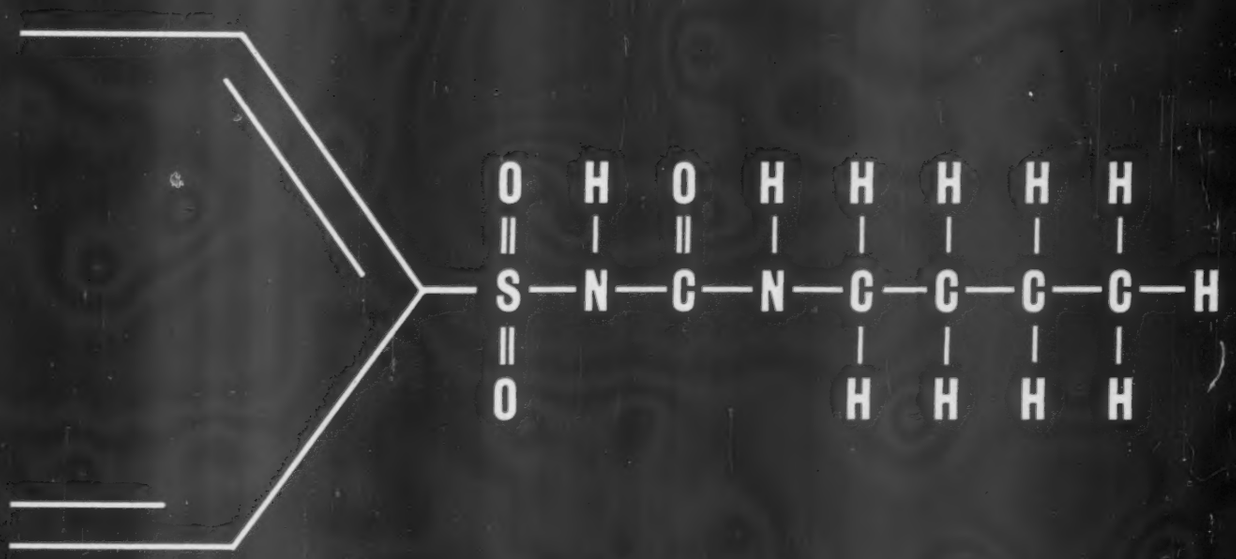

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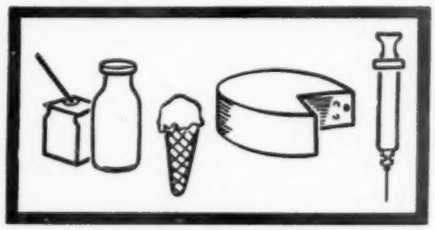

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†Zimmerman, M. C.: Clin. Med. *5*:305, 1958.

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2. Sherwood, H., and Cooke, R.A.: J. Allergy 28:97 (March) 1957.
3. Shelley, W.B.; Harun, J.S., and Pillsbury, D.M.: J.A.M.A. 167:959 (June 21) 1958.
4. Dubois, E.L.: California Med. 89:195 (Sept.) 1958.
5. Hartung, E.F.: J.A.M.A. 167:973 (June 21) 1958.

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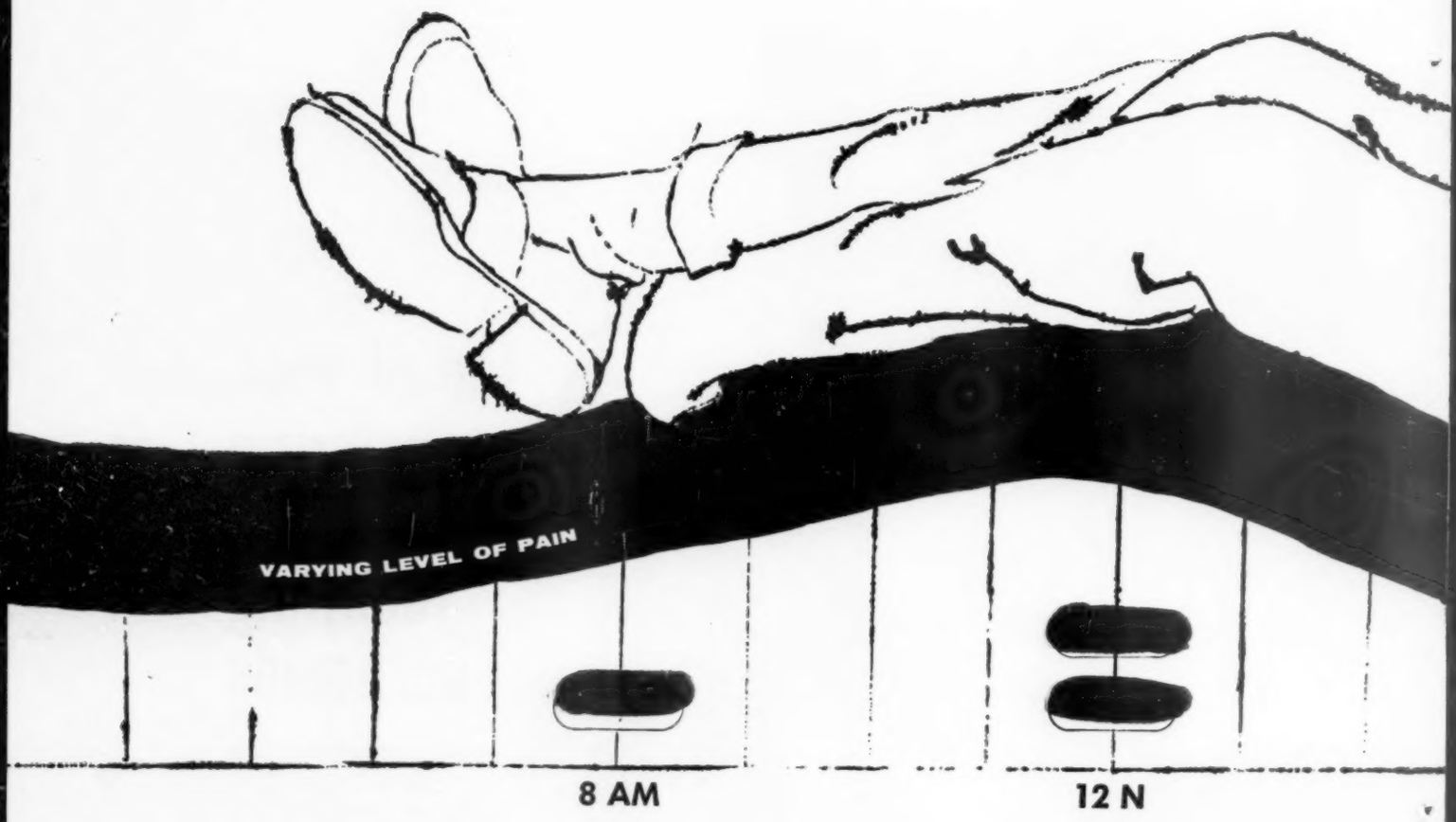

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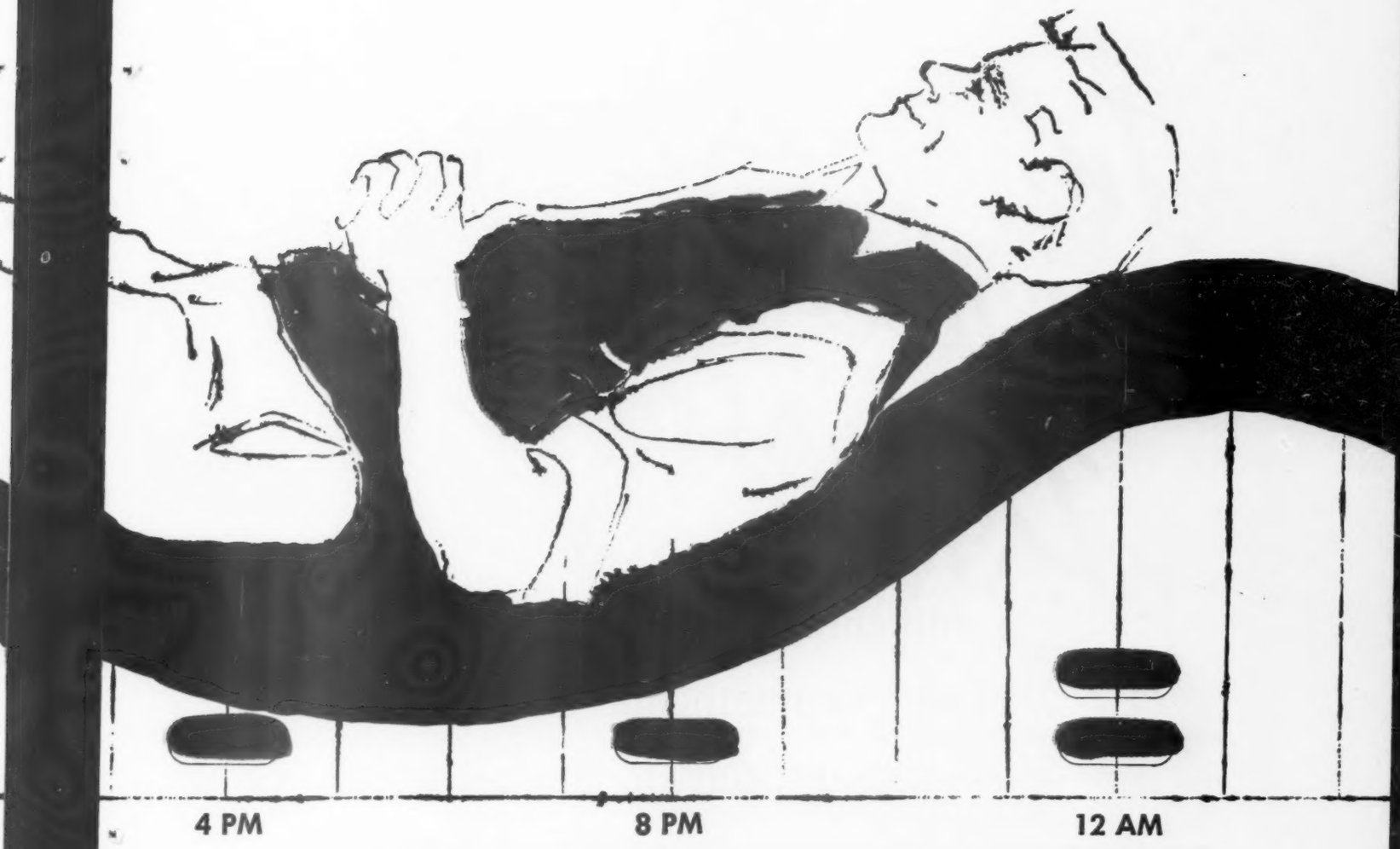

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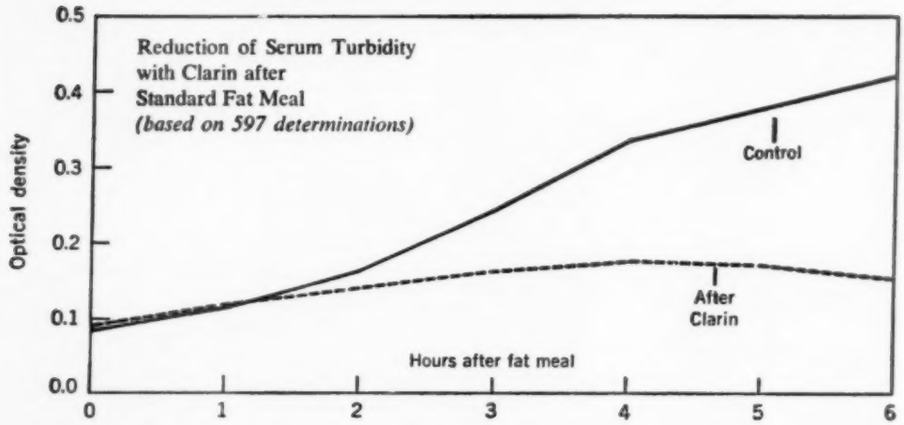

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1. Council on Drugs, J.A.M.A. *166*:52 (Jan. 4) 1958. 2. Hahn, P. F.: Science *98*:19 (July 2) 1943. 3. Fuller, H. L.: Angiology *9*:311 (Oct.) 1958. 4. Rubio, F. A., Jr.: Personal communication. 5. Engelberg, H., *et al.*: Circulation *13*:489 (April) 1956.

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1. Spies, T. D.: J.A.M.A. 167:675 (June 7) 1958.
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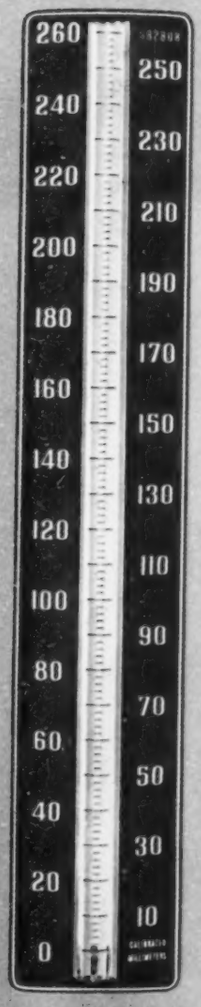

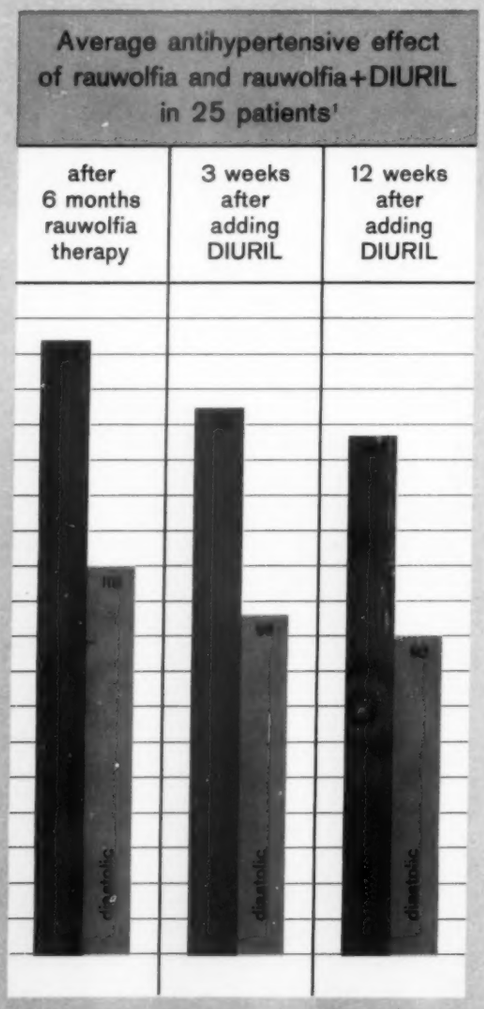
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diastolic
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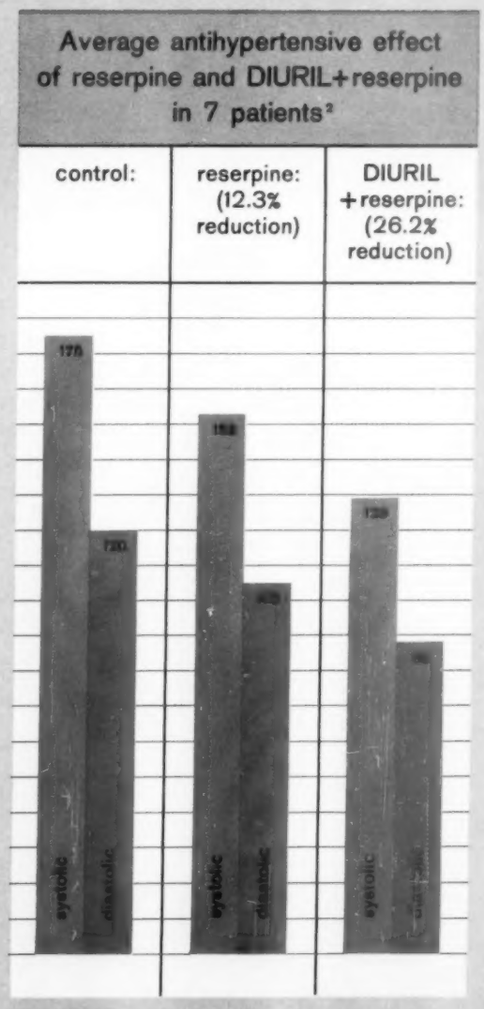

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diet more palatable

With DIUPRES, there is less need for rigid restriction of dietary salt, which patients find so burdensome.

> *"It may well be that the drug* [DIURIL] *produces the benefits of a markedly restricted low sodium diet but without its hardships."*[3]

subjective and objective improvement

DIUPRES allays anxiety and tension, thus reducing the emotional component of hypertension. Organic changes of hypertension may be arrested and reversed. Headache, dizziness, palpitations and tachycardia are usually promptly relieved by DIUPRES. When the *anginal syndrome* accompanies hypertension, the administration of DIUPRES may also cause diminution or even disappearance of this syndrome concurrent with control of the hypertension.

convenient, controlled dosage

Instead of two separate prescriptions, you write one prescription . . . the patient takes one tablet, rather than two different tablets . . . and the dosage schedule is easier for the patient to remember and follow.

> *"patients have fewer lapses and make fewer mistakes in dosage, the simpler the regimen can be made. Therefore I do not hesitate to use more than one medicament combined in one tablet, provided this gives approximately the correct dosage of each."*[6]

economical

DIUPRES will cost the patient less than if he were given two separate prescriptions for its components.

Indications:
DIUPRES is indicated in hypertension of all degrees of severity. It can be used in the following ways:

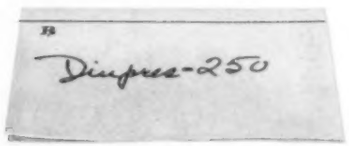
- as total therapy
- as primary therapy, adding other drugs if necessary
- as replacement or adjunctive therapy in patients now treated with other agents

Precautions:
The precautions normally observed with DIURIL or reserpine apply to DIUPRES. Additional information on DIUPRES is available to physicians on request.

Recommended dosage range:
DIUPRES-500—one tablet one to three times a day.
DIUPRES-250—one tablet one to four times a day.
If necessary, other agents may be added.
If the patient is receiving ganglion blocking agents or hydralazine, their dosage should be cut by 50 per cent when DIUPRES is added.

DIUPRES-500

500 mg. DIURIL (chlorothiazide), 0.125 mg. reserpine.
Bottles of 100, 1000.



DIUPRES-250

250 mg. DIURIL (chlorothiazide), 0.125 mg. reserpine.
Bottles of 100, 1000.

the first "wide range" antihypertensive

DIUPRES

DIURIL® WITH RESERPINE

1. Rochelle, J. B., III, Bullock, A. C., and Ford, R. V.: Potentiation of antihypertensive therapy by use of chlorothiazide, *J.A.M.A.* 168:410, Sept. 27, 1958. 2. Freis, E. D., Wanko, A., Wilson, I. M., and Parrish, A. E.: Treatment of essential hypertension with chlorothiazide (Diuril), *J.A.M.A.* 166:137, Jan. 11, 1958. 3. Freis, E. D.: Treatment of hypertension. (Presented at the Annual Meeting of Southern Medical Association, Nov. 13, 1957.) 4. Moyer, J. H., Dennis, E., and Ford, R.: Drug therapy (Rauwolfia) of hypertension, *A.M.A. Arch. Int. Med.* 96:530, Oct. 1955. 5. Perera, G. A.: Edema and congestive failure related to administration of rauwolfia serpentina, *J.A.M.A.* 159:439, Oct. 1, 1955. 6. Wilkins, R. W.: Precautions in use of antihypertensive drugs, including chlorothiazide, *J.A.M.A.* 167:801, June 14, 1958.

MSD MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

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Armour thyroid tablets assure: Consistent response—unsurpassed quality—highest manufacturing standards—full potency up to 17 years of storage—dependable therapy in: frank thyroid deficiencies and when hypothyroidism is associated with chronic recurrent colds, functional menstrual disorders, sterility, habitual abortion, obesity, hypometabolism. Thyroid is recommended in long-term therapy with ACTH or corticosteroids. Supplied in ¼, ½, 1, 2 and 5 grain strengths.

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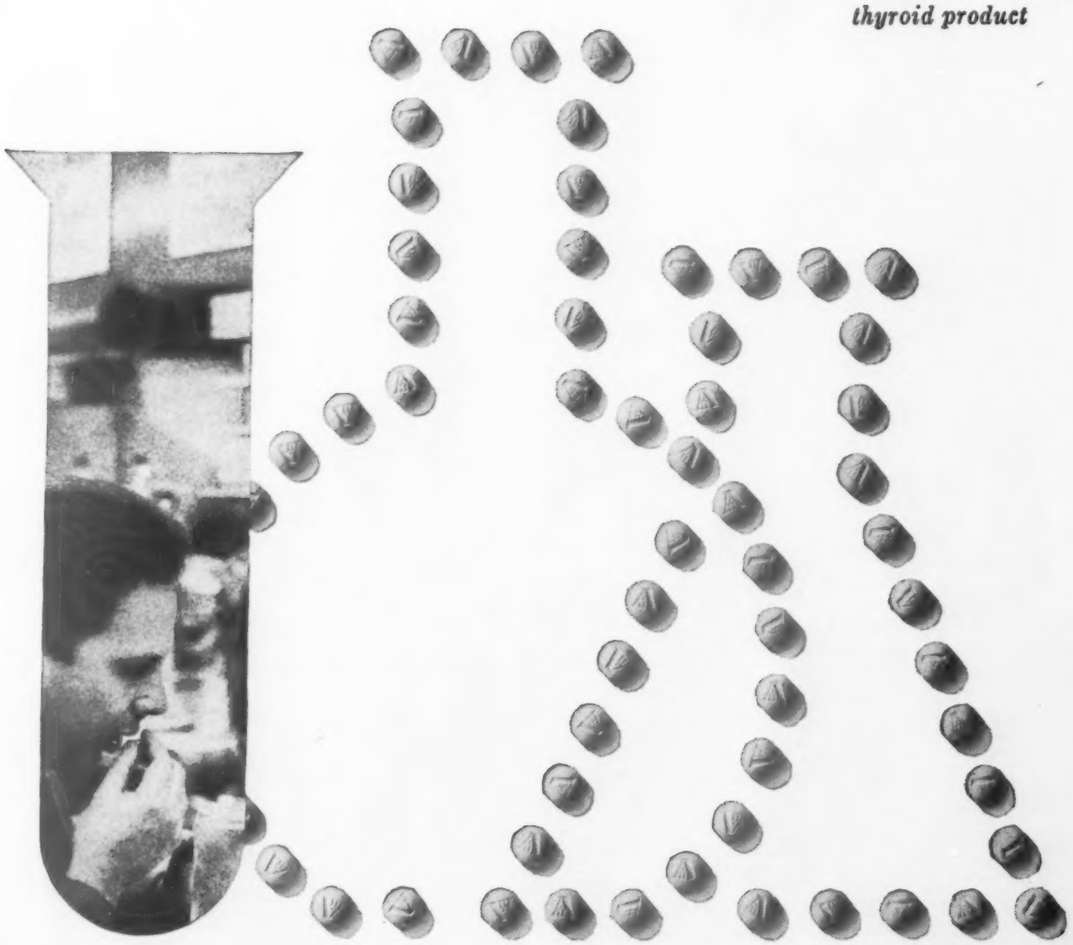

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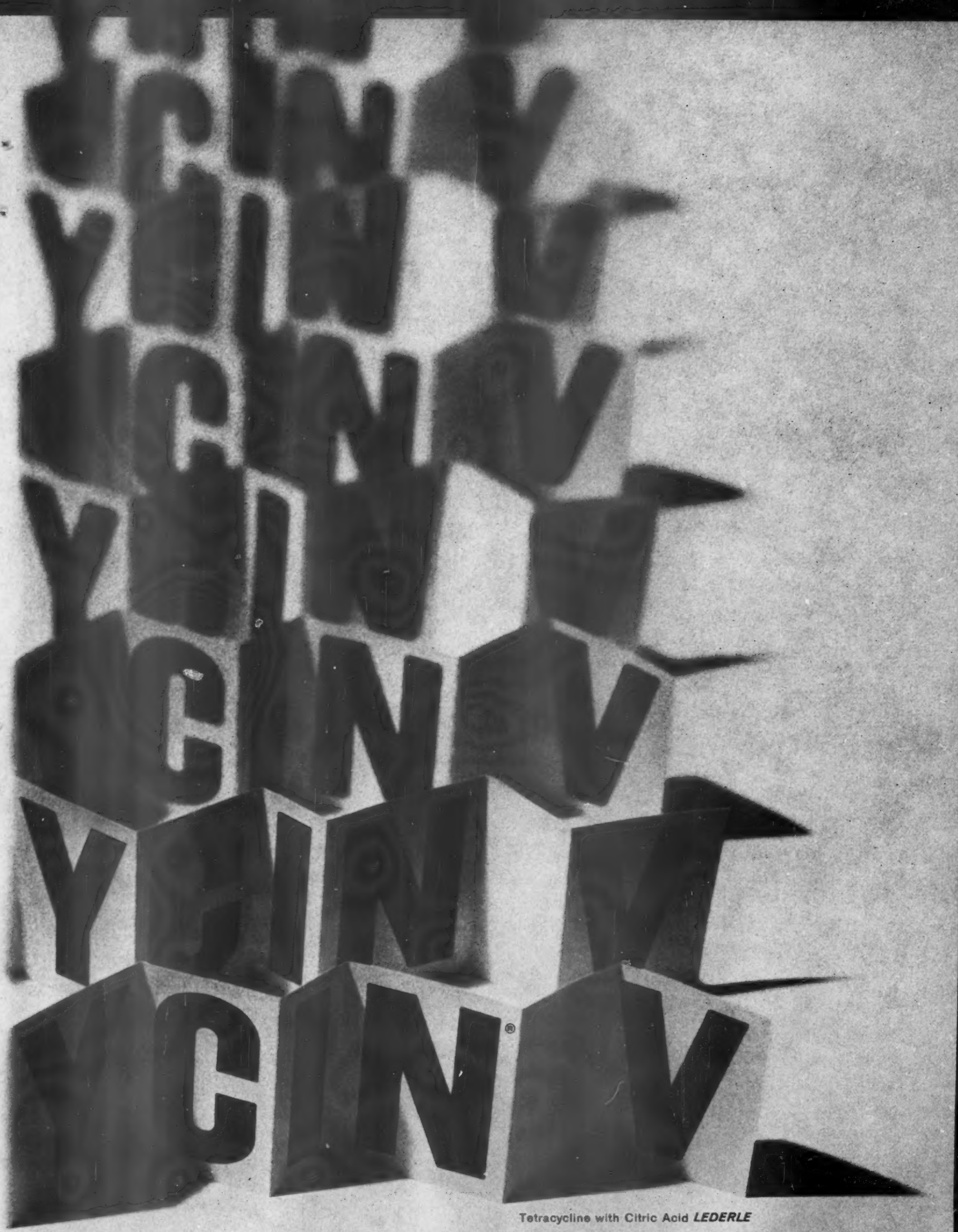

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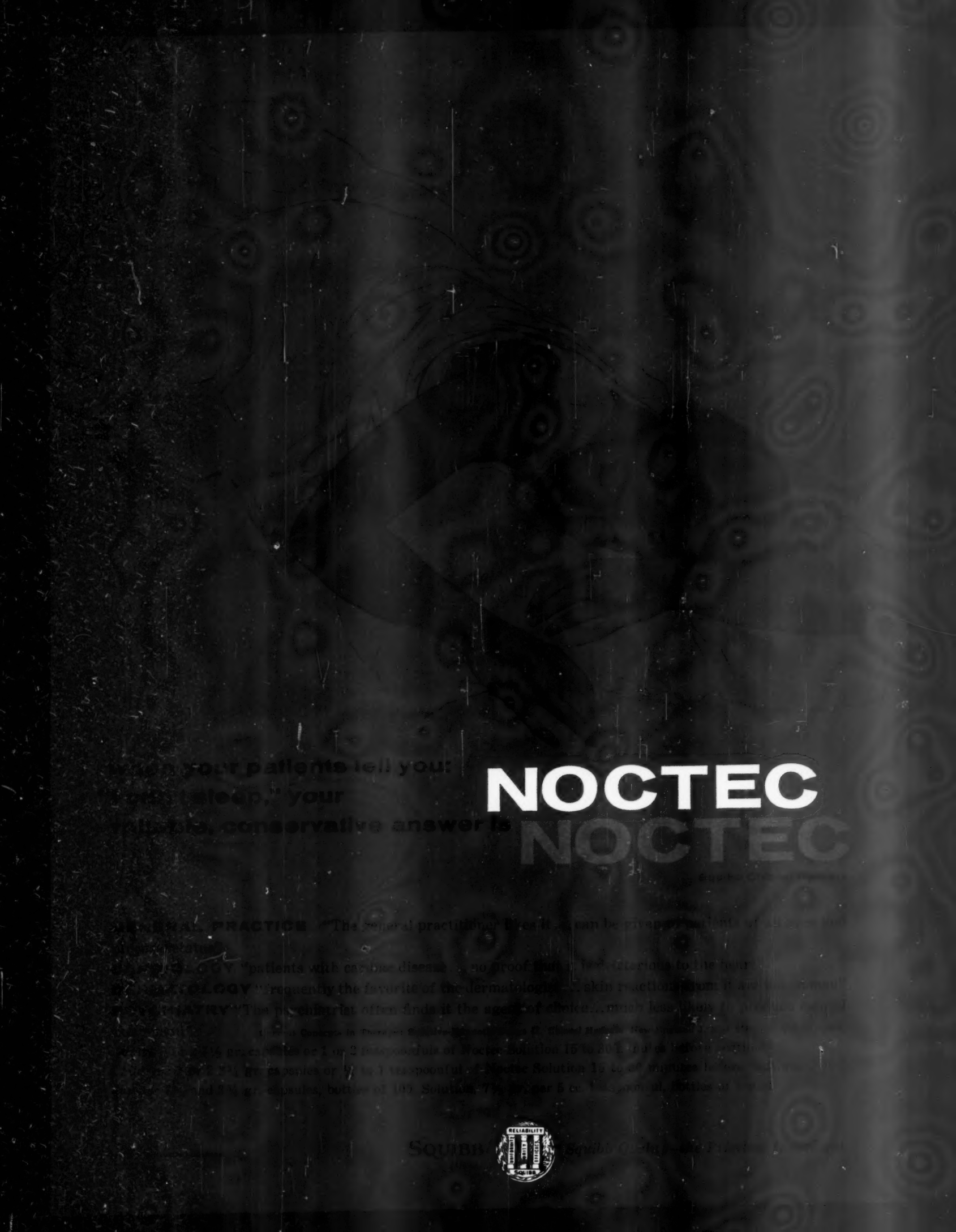
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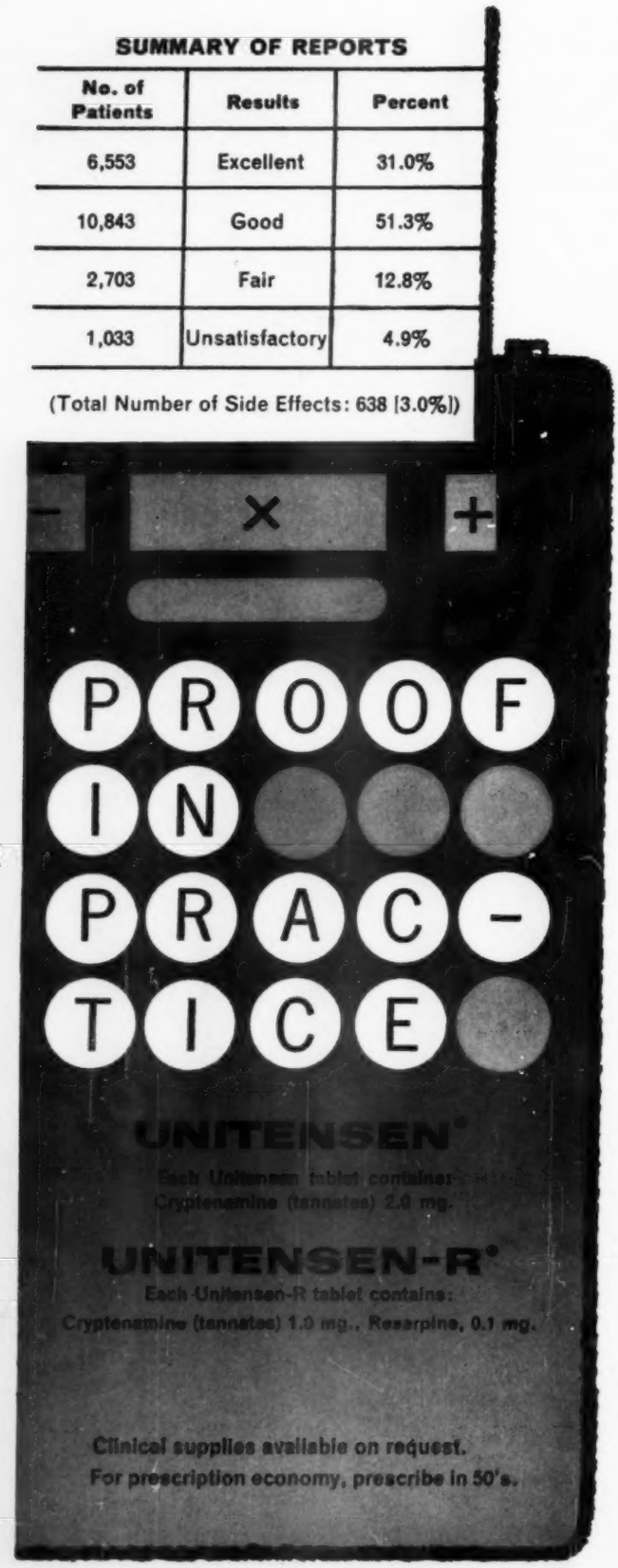
If you are not yet one of the many physicians who use 'Empirin' Compound with Codeine with satisfaction in your practice, I hope you will consider trying it soon.

Yours sincerely,

W. N. Creasy
President

P.S. Please let me know if you would like a bottle of <u>plain</u> 'Empirin' Compound for your family use.

*Acetophenetidin Gr. 2 1/2, Aspirin Gr. 3 1/2, Caffeine Gr. 1/2.



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This data deals with the results obtained by 1,988 physicians, treating 21,128 hypertensive patients with Unitensen. The "Proof In Practice" study validates, *in day-to-day private practice*, the findings of clinical trials conducted in hospitals and institutions. It proves that Unitensen affords safe, dependable office management for the majority of hypertensive patients. Unitensen lowers blood pressure . . . improves cerebral and renal blood flow... exerts no adverse effects on circulation . . . and, is virtually free of side effects.

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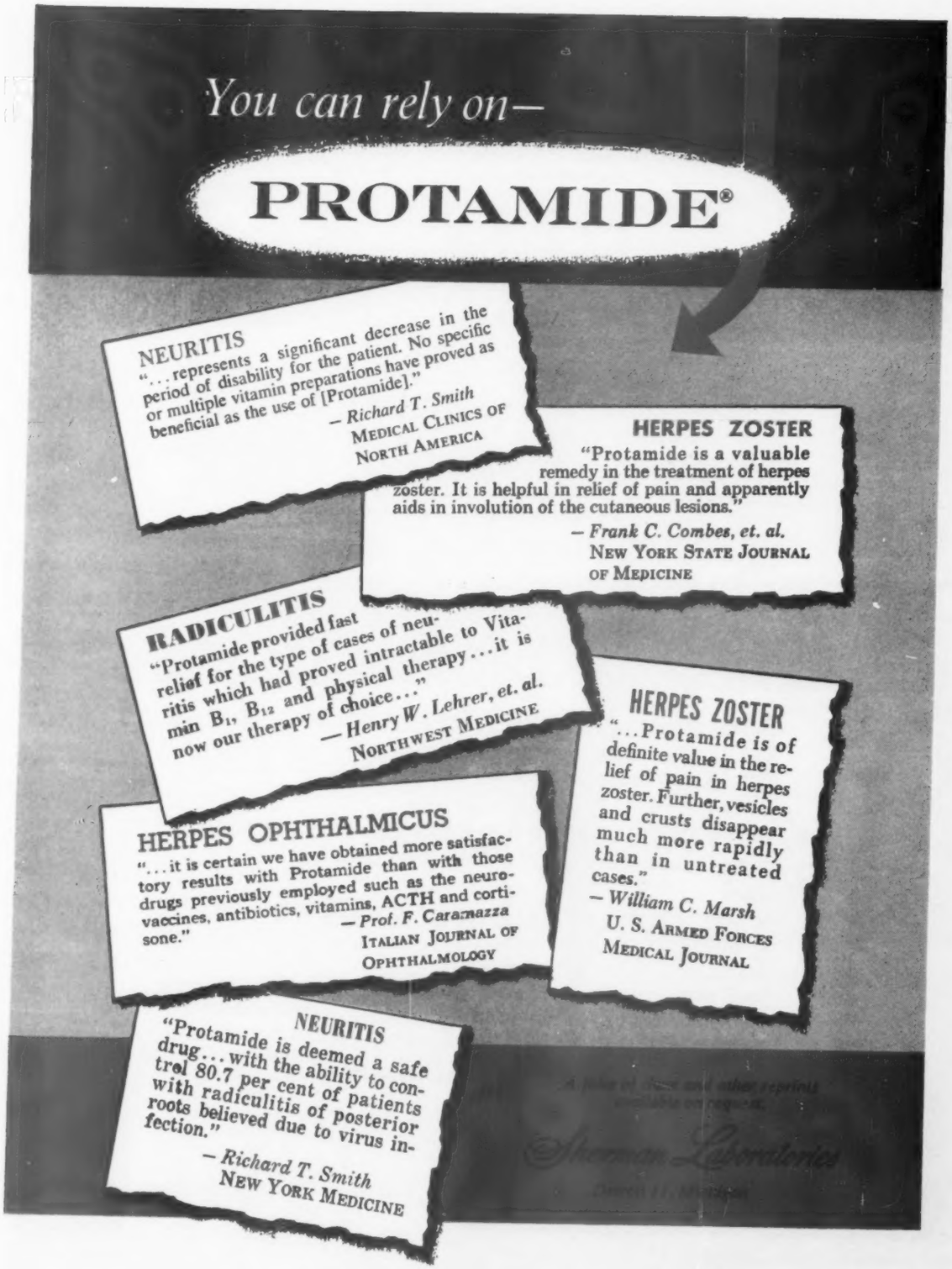
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"... represents a significant decrease in the period of disability for the patient. No specific or multiple vitamin preparations have proved as beneficial as the use of [Protamide]."
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"Protamide is a valuable remedy in the treatment of herpes zoster. It is helpful in relief of pain and apparently aids in involution of the cutaneous lesions."
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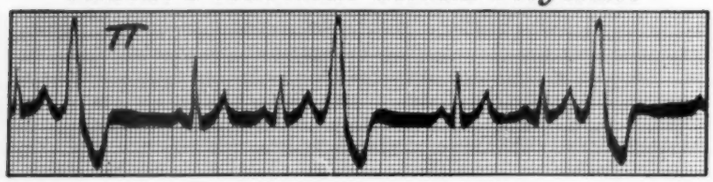
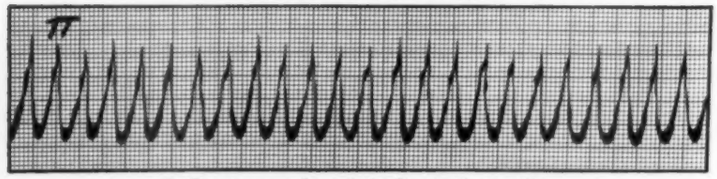
restore normal sinus rhythm



1. Premature ventricular contractions

2. Paroxysmal auricular tachycardia



3. Paroxysmal ventricular tachycardia

in arrhythmias

VISTARIL*

HYDROXYZINE PAMOATE

More than a tranquilizer

SPECIFIC ANTIARRHYTHMIC EFFECT

Vistaril is effective in ventricular extrasystoles and paroxysmal tachycardias (both auricular and ventricular).

plus

PSYCHOTHERAPEUTIC POTENCY

proven calming action indicated for arrhythmia patients.

and

THE OUTSTANDING SAFETY

of Vistaril as compared to other antiarrhythmic drugs in general use has been noted by investigators.

THE FOLLOWING DOSAGE REGIMEN IS RECOMMENDED

(individualized by the physician for maximum effectiveness):

PARENTERAL DOSAGE: 50-100 mg. (2-4 cc.) I.M. stat., and q. 4-6 h., p.r.n.; maintain with 25 mg. b.i.d. or t.i.d. In acute emergency, 50-75 mg. (2-3 cc.) I.V. stat.; maintain with 25-50 mg. (1-2 cc.) I.V. q. 4-6 h., p.r.n.

ORAL DOSAGE: Initially, 100 mg. daily in divided doses until arrhythmia disappears. For maintenance or prophylaxis, 50-75 mg. daily in divided doses.

SUPPLY: Vistaril Capsules, 25 mg., 50 mg. and 100 mg. Vistaril Parenteral Solution, 10 cc. vials and 2 cc. Steraject® Cartridges. Each cc. contains 25 mg. (as the hydrochloride).

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References: 1. Burrell, Z. I., et al.: Am. J. Cardiol., 1:624 (May) 1958. 2. Hutcheon, D. E., et al.: J. Pharmacol. & Exper. Therap., 118:451 (Dec.) 1956.

During her reproductive years— almost every woman needs iron therapy

The average woman loses 1 mg. of iron per day through normal elimination. Add to this an average of 15 to 30 mg. each menstrual period.[1] It is evident that many women deplete their iron reserves faster than the normal diet can replenish them.

Many clinicians recommend that every woman, during her reproductive years, receive iron therapy for six weeks each year.

Livitamin, with *peptonized* iron, offers an excellent formula to restore depleted iron reserves. Peptonized iron is well absorbed and stored, and less irritating than other forms. The Livitamin formula, which contains the B complex, provides integrated therapy to normalize the blood picture.

1. Brown, E.B., Jr. *The Management of Iron Deficiency Anemia*, GP, 2:87 (Feb. 1958).

LIVITAMIN®

with Peptonized Iron

FORMULA: Each fluidounce contains:

Iron peptonized	420 mg.
(Equiv. in elemental iron to 71 mg.)	
Manganese citrate, soluble	158 mg.
Thiamine hydrochloride	10 mg.
Riboflavin	10 mg.
Vitamin B_{12} Activity	20 mcg.
(derived from Cobalamin conc.)	
Nicotinamide	50 mg.
Pyridoxine hydrochloride	1 mg.
Pantothenic acid	5 mg.
Liver fraction 1	2 Gm.
Rice bran extract	1 Gm.
Inositol	30 mg.
Choline	60 mg.

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Livitamin is better because it contains peptonized iron

current studies show peptonized iron*

- **Absorbed as well as ferrous sulfate.**
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- **Free from tendencies to disturb digestion. (One-tenth as irritating to the gastric mucosa as ferrous sulfate.)**
- **More rapid response in iron-deficient anemias.**
- **One-third as toxic as ferrous sulfate.**

*Keith, J.H.: Utilization and Toxicity of Peptonized Iron and Ferrous Sulfate, Am. J. Clin. Nutrition *1*:35 (Jan.-Feb., 1957).

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FOR RHEUMATISM AND TRAUMATIC DISORDERS

PARAFON*

THE SPECIFIC MUSCLE RELAXANT PLUS
THE PREFERRED ANALGESIC

FOR ARTHRITIS

PARAFON* with PREDNISOLONE

Effective and well tolerated on the practical dosage of only 6 tablets daily, PARAFON and PARAFON WITH PREDNISOLONE provide benefits that last for up to six hours.

PARAFON relieves pain, stiffness, and disability caused by rheumatism and traumatic disorders; PARAFON WITH PREDNISOLONE compounds this relief with anti-inflammatory action in treatment for arthritis.

supplied: PARAFON: Tablets, scored, pink, bottles of 50. Each tablet contains: PARAFLEX Chlorzoxazone† 125 mg.; and TYLENOL® Acetaminophen 300 mg.
PARAFON WITH PREDNISOLONE: Tablets, scored, buff colored, bottles of 36. Each tablet contains: PARAFLEX Chlorzoxazone 125 mg.; TYLENOL Acetaminophen 300 mg.; and prednisolone 1.0 mg.

precautions: The precautions and contraindications that apply to all steroids should be kept in mind when prescribing PARAFON WITH PREDNISOLONE.

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Ethoheptazine Citrate with Acetylsalicylic Acid, Wyeth

for everyday pain control . . .

for your many patients requiring potent analgesia but not an injected narcotic

Proved by extensive evaluation[1,2,3] in 1998 patients in diverse areas of medicine and surgery, including:

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- *exclusive Wyeth non-narcotic analgesic plus anti-inflammatory action*
- *prompt, potent action—as potent as codeine*
- *documented effectiveness and safety[1,2,3]*

Supplied: Tablets, bottles of 48. Each tablet contains 75 mg. of ethoheptazine citrate and 325 mg. (5 grains) of acetylsalicylic acid.

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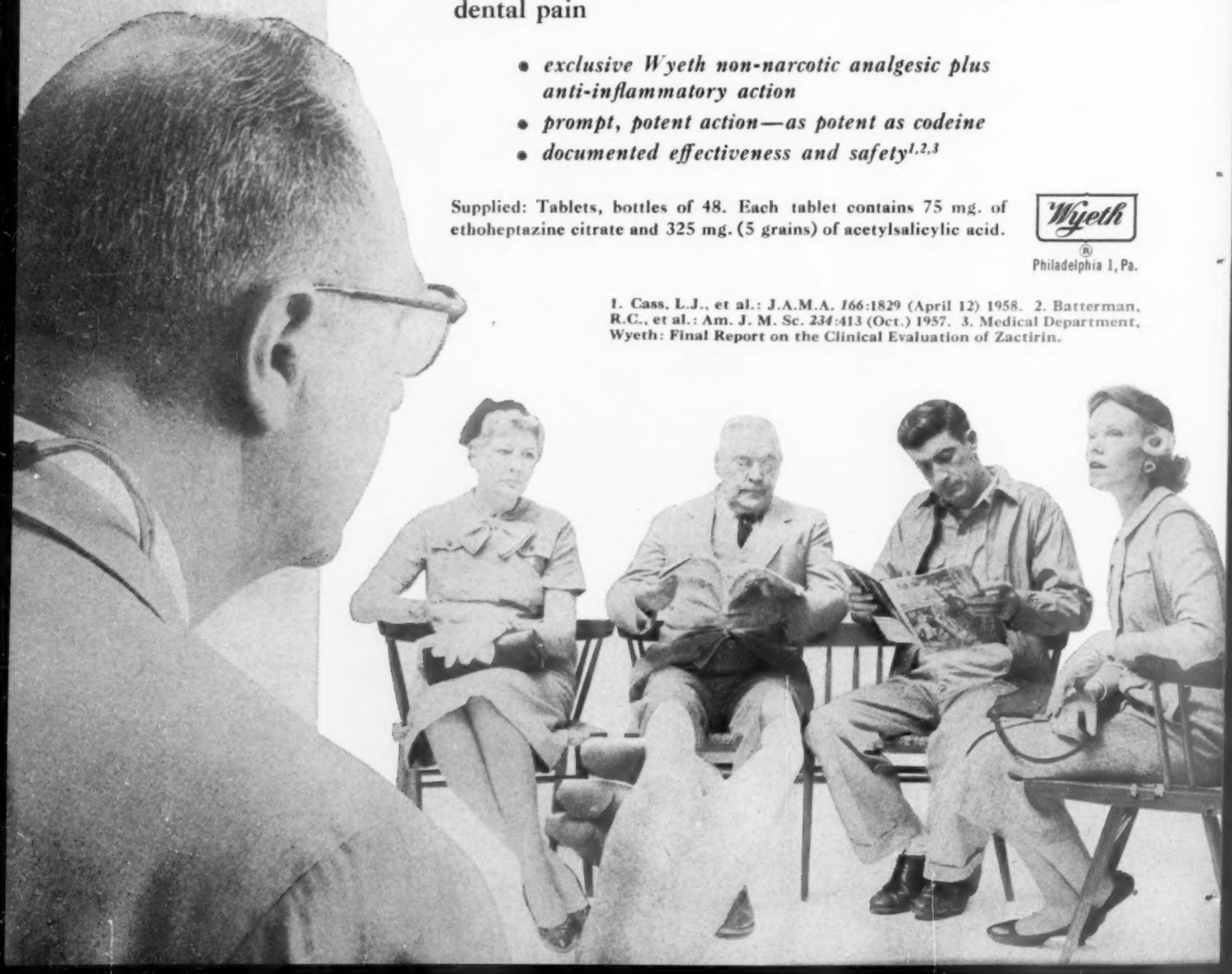
1. Cass, L.J., et al.: J.A.M.A. *166*:1829 (April 12) 1958. 2. Batterman, R.C., et al.: Am. J. M. Sc. *234*:413 (Oct.) 1957. 3. Medical Department, Wyeth: Final Report on the Clinical Evaluation of Zactirin.



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Operation is fully automatic with selective sterilizing cycles from 3 to 60 minutes. Cools and dries instruments or supplies by exhausting steam and residual water back into water reservoir—NOT into room.

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Psychic sedative
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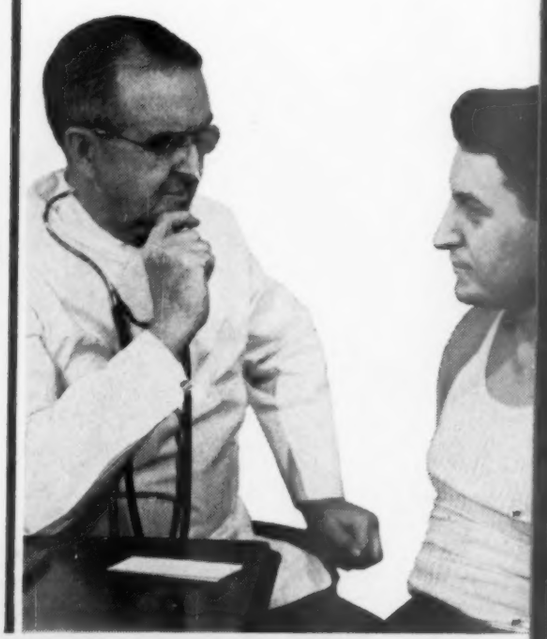

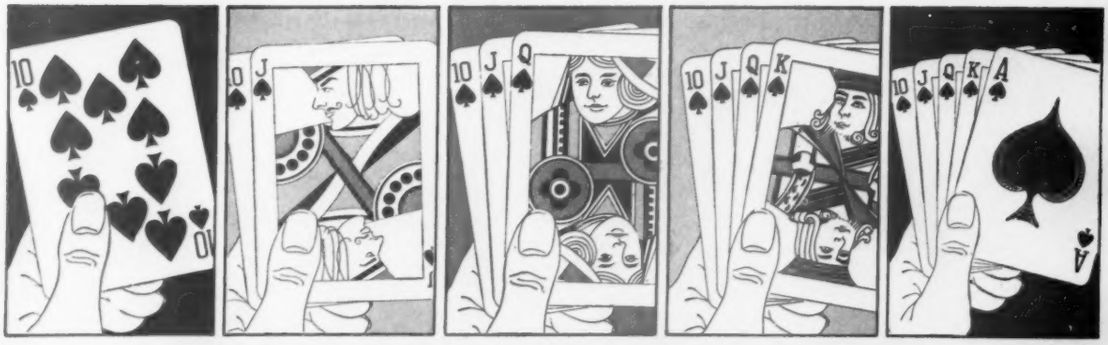

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cough sedative / antihistamine / expectorant

- relieves cough and related symptoms in 15-20 minutes
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- rarely constipates • cherry-flavored

Each teaspoonful (5 cc.) of HYCOMINE contains:

Hycodan®		
Dihydrocodeinone Bitartrate (Warning: May be habit-forming)	5 mg.	6.5 mg.
Homatropine Methylbromide	1.5 mg.	
Pyrilamine Maleate		12.5 mg.
Ammonium Chloride		60 mg.
Sodium Citrate		85 mg.

Adult Dosage: one teaspoonful q. 6 h. May be habit-forming.
Federal law permits oral prescription.

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The drug that lowered this patient's blood pressure *for the first time without side effects* is now available for your prescription...

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Singoserp™
(syrosingopine CIBA)

here is the full story... *please turn*

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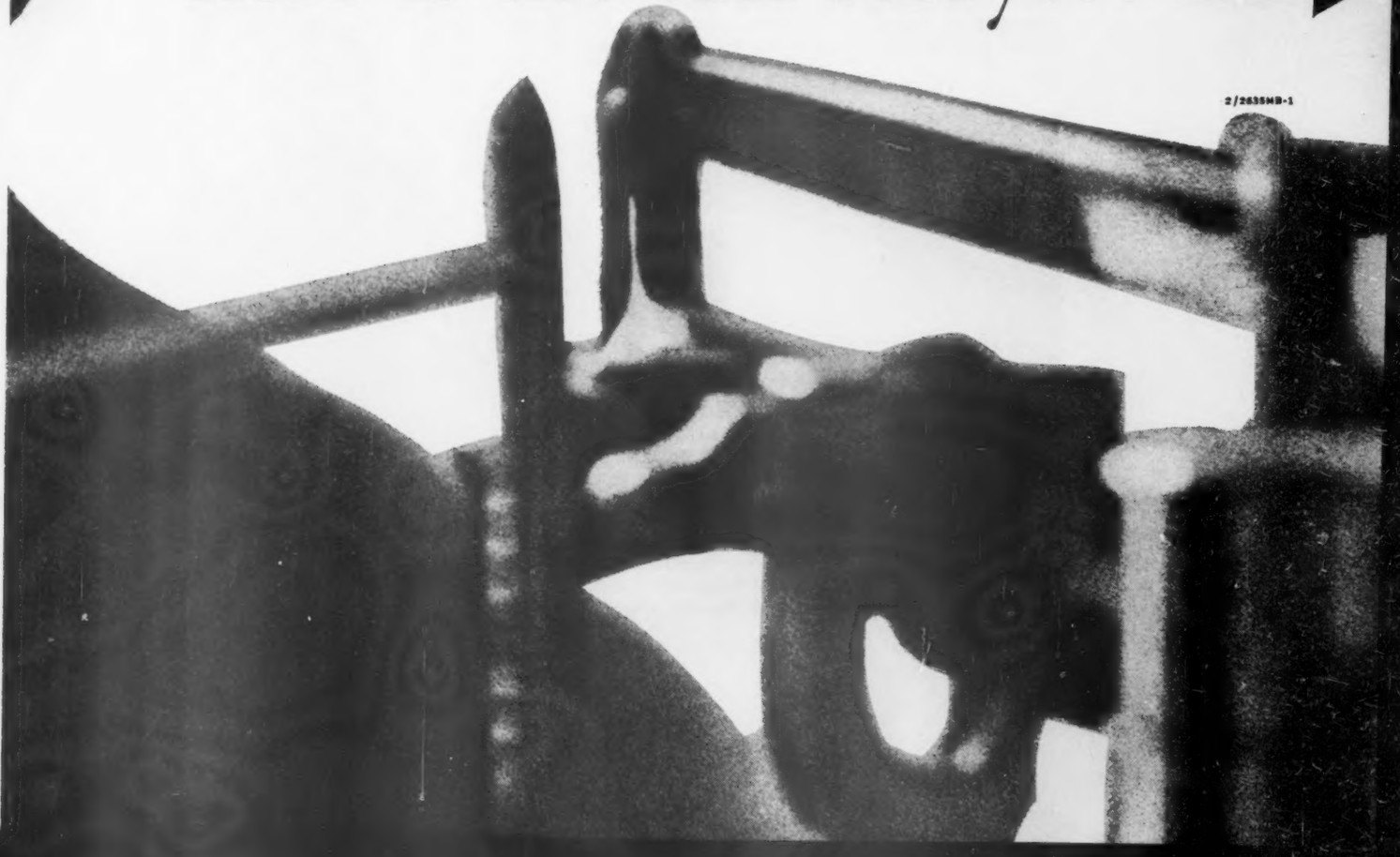


a major improvement in rauwolfia
a major advance in antihypertensive therapy

Developed after three years of basic research, proved during one of the most extensive clinical trials in pharmaceutical history, here is what **Singoserp** *can do:*

Patient P. K. was first seen with a blood pressure of 220/138 mm. Hg; he complained of headache, palpitation, nervous tension and hyperhidrosis.

Hospitalized briefly for observation and treatment, he was placed on a 4-Gm. sodium diet, plus chlorothiazide and mecamylamine regulated according to b.p. reading, which he was taught to take himself.

One month later his blood pressure was 140/104; he complained of dryness of mouth, chest pain, constipation and nocturia (twice a night). He was then started on Singoserp (0.5 mg. daily) with instructions to reduce the other medications to the extent possible, as evidenced by his b.p. readings.

After five months on Singoserp the patient's blood pressure ranged between 120/84 and 140/100. No mecamylamine was required; only ⅓ the original dose of chlorothiazide was required. One month later, chlorothiazide was stopped and the patient was maintained on Singoserp alone, 1 mg. b.i.d. Favorable blood pressure response continues and patient feels well. Since taking Singoserp patient reports *no* chest pain, *no* mouth dryness, *no* other side effects.

Singoserp T.M.

(syrosingopine CIBA)

Solves the Side Effects Problem in Most Hypertensive Patients

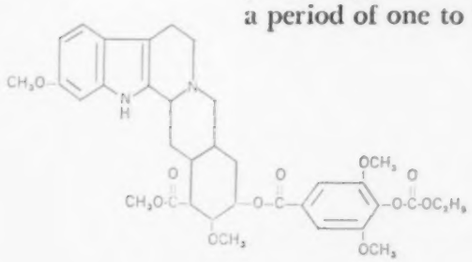
1. For new hypertensive patients Singoserp is the ideal antihypertensive drug for new patients because it lowers blood pressure without creating the side effects problem posed by conventional rauwolfia agents.

2. For hypertensive patients already undergoing drug treatment Singoserp, added to any antihypertensive regimen, makes it possible to maintain blood pressure levels achieved with more potent agents, while reducing their dosage requirements—or even eliminating them altogether in some cases.

Infrequent side effects —"The chief advantage of [Singoserp] over other Rauwolfia derivatives seems... to be the relative infrequency with which it produces disturbing side effects."[1]

Less sedation—"It [Singoserp] is approximately equipotent to reserpine as a hypotensive agent but is definitely less sedative or tranquilizing."[2]

Depression relieved —"In those patients who had been depressed, [Singoserp] was substituted for other Rauwolfia preparations and within a period of one to two weeks this depression was relieved."[3]



Created in the laboratory by altering the reserpine molecule so as to preserve its antihypertensive property and virtually eliminate its undesirable side actions.

Dosage: *In New Patients:* Average initial dose, 1 to 2 tablets (1 to 2 mg.) daily. Some patients may require and will tolerate 3 or more tablets daily. Maintenance dose will range from ½ to 3 tablets (0.5 mg. to 3 mg.) daily. When necessary for adequate control of blood pressure, more potent agents may be used adjunctively with Singoserp in doses below those required when they are used alone. *In Patients Taking Other Antihypertensive Medication:* Add 1 to 2 Singoserp tablets (1 to 2 mg.) daily. Dosage of other agents should be revised downward to a level affording maximal control of blood pressure and minimal side effects.

Supplied: Singoserp *Tablets,* 1 mg. (white, scored); bottles of 100.

References: 1. Herrmann, G. R., Vogelpohl, E. B., Hejtmancik, M. R., and Wright, J. C.: To be published. 2. Wolffe, J. B.: Mod. Med. *26*:253 (Feb. 1) 1958. 3. Bartels, C. C.: To be published.

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SUMMIT, N. J.

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Meprobamate and Pentaerythritol Tetranitrate

Tablets, vials of 50, meprobamate (200 mg.) and pentaerythritol tetranitrate (10 mg.)

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CLISTIN® EXPECTORANT

—with its antitussive-antihistaminic-expectorant actions—tackles coughs that "hang on" after colds—coughs of allergic or non-allergic origin.

CLISTIN EXPECTORANT is pleasantly fruit flavored, lemon-yellow colored, non-narcotic—does not upset the stomach.

Each 5 cc. contains: Clistin® Carbinoxamine Maleate 2 mg., Ammonium Chloride 120 mg., Sodium Citrate 120 mg., Potassium Guaiacolsulfonate 60 mg., Chloroform 0.01 cc., Benzyl Alcohol 0.3 %

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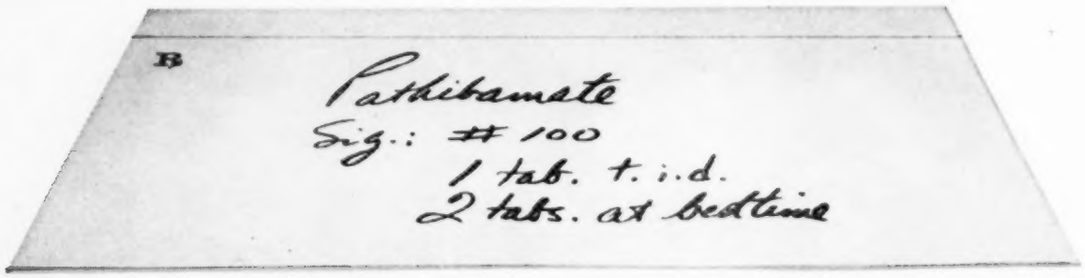

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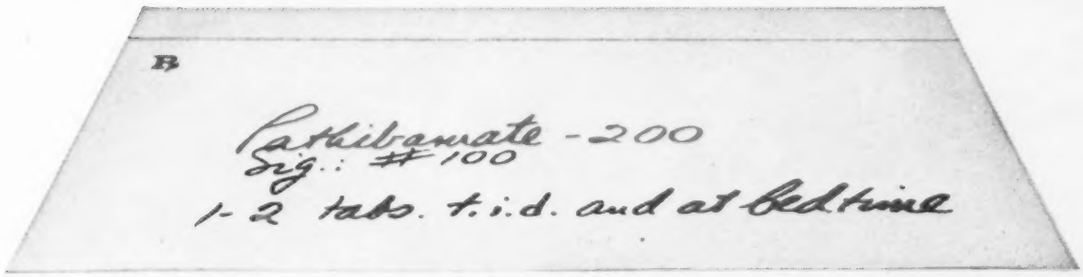
NEW! for greater flexibility in the control of tension and G.I. trauma... now you can write:



In the management of such gastrointestinal dysfunctions as duodenal or gastric ulcer, intestinal colic, spastic and irritable colon, ileitis, esophageal spasm, gastric hypermotility and anxiety neurosis with G. I. symptoms, nearly two years' experience has confirmed the clinical advantages derived from the combination of the two agents in PATHIBAMATE.

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With PATHIBAMATE-200, further individualization of treatment is facilitated in respect to both the degree of tension and associated G.I. sequelae, as well as the response of different patients to the component drugs.

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Administration and dosage: PATHIBAMATE-400 — 1 tablet three times a day and 2 tablets at bedtime.
PATHIBAMATE-200 — 1-2 tablets three times a day and at bedtime. Adjust dosage to patient response.

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Meprobamate with PATHILON® Tridihexethyl Chloride* LEDERLE

*PATHILON is now offered as tridihexethyl chloride instead of the iodide, since the latter may interfere with the results of certain thyroid function tests.

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► restores natural sleep

► reduces depressive rumination and crying

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Deprol is unlike central nervous stimulants

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Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this dose may be gradually increased up to 3 tablets q.i.d.

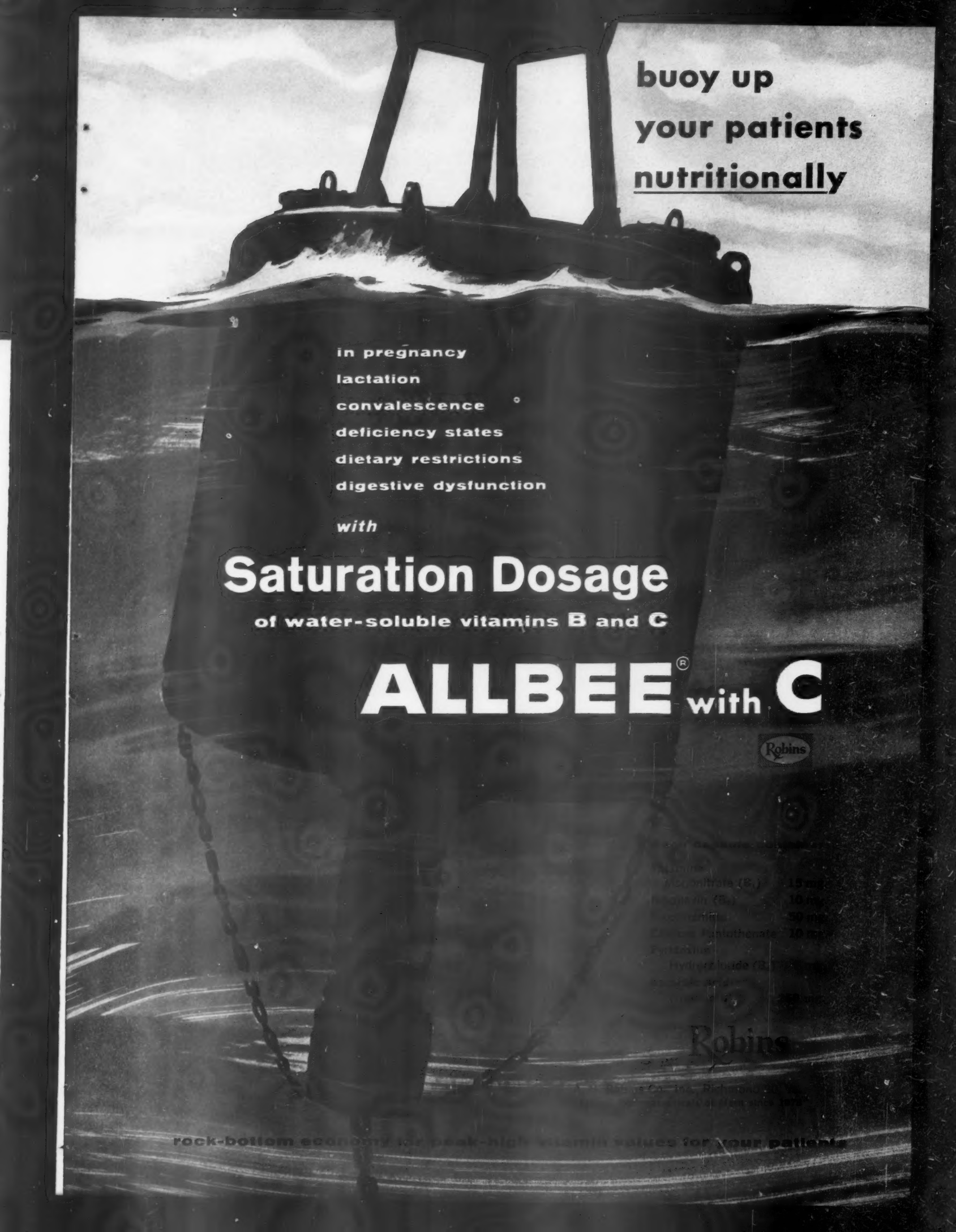
Composition: Each tablet contains 400 mg. meprobamate and 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl).

Supplied: Bottles of 50 scored tablets.

1. Alexander, L.: Chemotherapy of depression—Use of meprobamate combined with benactyzine (2-diethylaminoethyl benzilate) hydrochloride. J.A.M.A. 166:1019, March 1, 1958. 2. Current personal communications; in the files of Wallace Laboratories.

†TRADE MARK (D-749)

Literature and samples on request WALLACE LABORATORIES, *New Brunswick, N.J.*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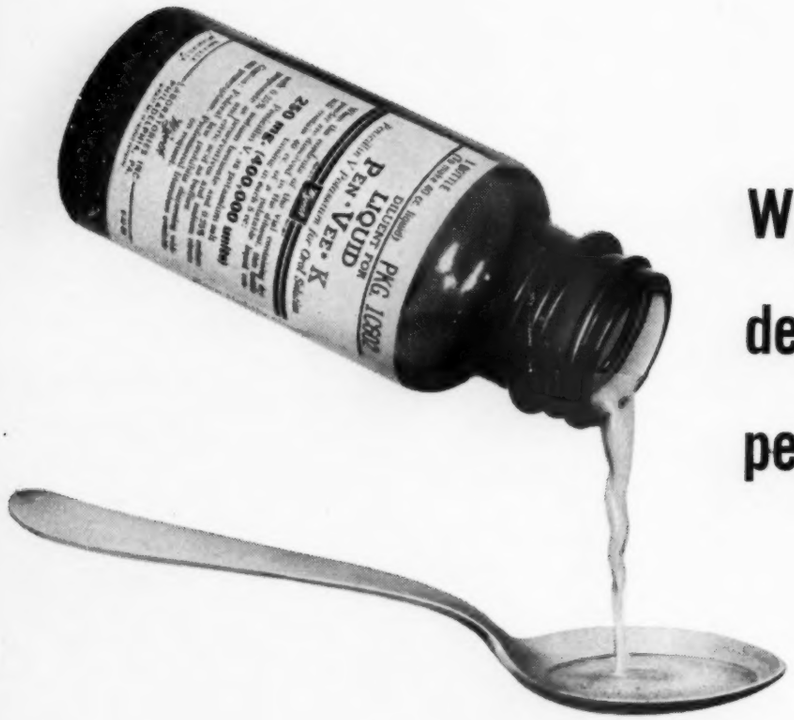

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REFERENCES: 1. Carlozzi, M.: Antibiotic Med. & Clin. Therapy 5:146 (Feb.) 1958. 2. Welch, H.; Wright, W. W., and Staffa, A. W.: Antibiotic Med. & Clin. Therapy 5:52 (Jan.) 1958. 3. Marlow, A. A., and Bartlett, G. R.: Glucosamine and leukemia, Proc. Soc. Exp. Biol. & Med. 84:41, 1953. 4. Shalowitz, M.: Clin. Rev. 1:25 (April) 1958. 5. Nathan, L. A.: Arch. Pediat. 75:251 (June) 1958. 6. Cornbleet, T.; Chesrow, E., and Barsky, S.: Antibiotic Med. & Clin. Therapy 5:328 (May) 1958. 7. Stone, M. L.; Sedlis, A., Bamford, J., and Bradley, W.: Antibiotic Med. & Clin. Therapy 5:322 (May) 1958. 8. Harris, H.: Clin. Rev. 1:15 (July) 1958.

- thank you, doctor"

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Antibacterial effectiveness plus added protection against monilial superinfection

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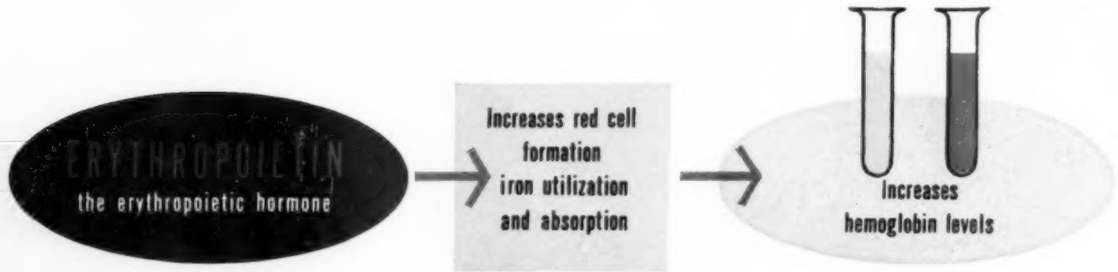

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in less than five months, record of squeeze-bulb test shows repetition frequency increased from 31 to 50 consecutive times in left hand—much improved in right.*

*SQUEEZE-BULB MUSCLE ENDURANCE TEST

Patient A.W., female, age 71

"Mediatric" therapy started at time of first test and continued for 4½ months		Repetition frequency (consecutive times)	
		Left hand	Right hand
1st test	Feb. 16, 1954	31	58
2nd test	July 1, 1954	50	67

improved muscle endurance with steroid-nutritional therapy objectively demonstrated by physical tests

Muscle strength and endurance were determined by stiff-bulb test in a series of older patients before and after "Mediatric" therapy. Improvement was measured by the increase in the number of times a patient could squeeze the stiff bulb at a steady rhythm. Results of this test showed greatly enhanced muscle endurance after continuous "Mediatric" therapy, even in short periods of time.[1] During this period, grip strength of these same patients was measured with a dynamometer, and joint range with a goniometer. These and other physical tests also showed remarkable increase in strength, endurance and coordination.

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"Mediatric" contains estrogen and androgen in amounts that will help counteract declining gonadal hormone secretion, maintain a positive nitrogen balance, and promote synthesis of protein in muscle, bone and other tissues.

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For better health and vigor in the older patient

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STEROID-NUTRITIONAL COMPOUND

each capsule or tablet contains:

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Conjugated estrogens equine ("Premarin"®)	0.25	mg.
Methyltestosterone	2.5	mg.
NUTRITIONAL SUPPLEMENTS		
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Vitamin B_{12} with intrinsic factor concentrate	1/6	U.S.P. Unit
Thiamine mononitrate (B_1)	10.0	mg.
Riboflavin (B_2)	5.0	mg.
Nicotinamide	50.0	mg.
Pyridoxine HCl (B_6)	3.0	mg.
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d-Desoxyephedrine HCl	1.0	mg.

Suggested Dosages: Male — *1 capsule or 1 tablet daily, or as required.* Female — *1 capsule or 1 tablet daily, or as required, taken in 21 day courses with a rest period of one week between courses.*

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Also available: *"Mediatric" Liquid — No. 910 — Bottles of 16 fluidounces and 1 gallon.*

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1. Perlman, R. M., and Dorinson, S. M.: Presented before the Third Congress of the International Association of Gerontology, London, England, July 19-23, 1954.

in peptic ulcer

REFRACTORY CASES RESPOND TO NEW

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OXYPHENCYCLIMINE HYDROCHLORIDE

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curbs secretion when excessive
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Potency and Prolonged Duration of Action
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References: 1. Finkelstein, Murray: Journal of Pharmacology and Experimental Therapeutics, in press. 2. Winkelstein, Asher: Paper in preparation.

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THE RATIONALE FOR THE USE OF VITAMINS IN FORESTALLING INFECTIONS

Many clinicians believe that good nutrition plays a significant role in preventing bacterial infections, and that immunity depends on adequate vitamin levels. Tisdall[1] states that "a low intake of a number of vitamins, a low intake of minerals, and a change in the quality of protein can all lower resistance to infection."

Other studies show the important role of the B vitamins in antibody formation. Thus, *Nutrition Reviews*[2] reports: "Present evidence indicates that certain B vitamins, notably pyridoxine, pantothenic acid and folacin, play a significant role in antibody synthesis." According to Pollack and Halpern,[3] "Under-nutrition leads to increased susceptibility to infection and decreased resistance to established disease." And "vitamin deficiency states also may adversely influence circulating antibodies."

Halpern[4] reports that "good nutrition is important for optimal resistance to infection, for a superior tissue capability to cope with disease and injury, and for maximum antibody production . . . nutrition participates in the prophylaxis against most acute infections . . ."

And while MacBryde[5] feels that evidence is lacking to support the view that a higher than normal intake of vitamins will improve resistance to infection, he also states: "Restoration of nutrition to normal exerts a favorable influence on practically all disease conditions . . . Often the outcome will depend more upon the correction of the malnutrition than upon any therapy directed toward the malady."

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now expanded to include additional essential vitamins—

and at no extra cost to your patients

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Vitamin A	25,000 U.S.P. units
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Thiamine Mononitrate	10 mg.
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Vitamin B_{12} Activity Concentrate	5 mcg.

Dosage: 1 or more capsules daily as indicated.

Supply: Family Packs of 180. Bottles of 30, 60, 100 and 1,000.

Also Available: THERAGRAN Liquid, bottles of 4 ounces; THERAGRAN Junior bottles of 30 and 100 capsules; and THERAGRAN-M (Squibb Vitamin-Minerals for Therapy), bottles of 30, 60, 100 and 1,000 capsule-shaped tablets.

References: 1. Tisdall, F. F.: Clinical Nutrition, ed. by Joliffe, N.; Tisdall, F. F., and Cannon, P. R.: Paul B. Hoeber, Inc., New York, 1950, p. 748. 2. Nutrition Reviews, *15*:47, (Feb.) 1957. 3. Pollack, H., and Halpern, S. L.: Therapeutic Nutrition, National Academy of Sciences and National Research Council, Washington, D. C., 1952, p. 18. 4. Halpern, S. L.: Ann. N. Y. Acad. Science *63*:147, (Oct. 28) 1955. 5. MacBryde, C. N.: Signs and Symptoms, J. B. Lippincott Co., Phila., 3rd Ed. 1957, p. 818.

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1. Kirsner, J.B., et al.: M. Clin. North America *41*:499 (March) 1957.

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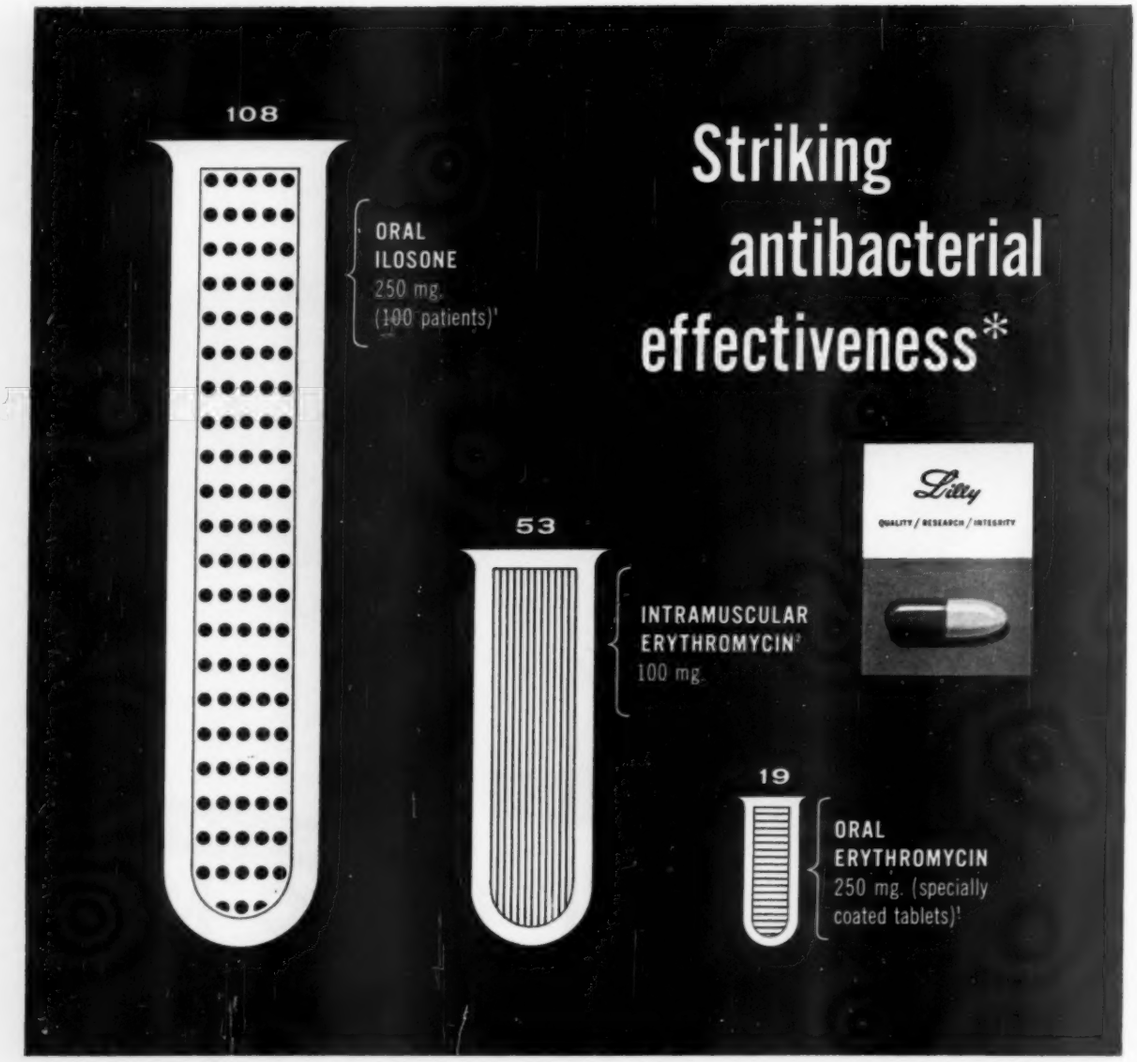

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*Shown by *how many times* the serum can be diluted two hours after administration of the antibiotic and still inhibit identical pathogenic strains of bacteria. This is the *Tube Dilution Technique*, which is regarded by leading authorities as the most meaningful method of comparing different antibiotics. It shows not merely the level of antibiotic in the blood but the actual antibacterial effectiveness of that level.

1. Griffith, R. S., *et al.:* Antibiotic Med. & Clin. Therapy, *5*:609 (October), 1958. Note: Peak levels with the oral erythromycin tablets (thirty-three dilutions) were not observed until four hours after administration. 2. Data from Griffith, R. S.: Antibiotics Annual, p. 269, 1954-1955.

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The American Journal of Medicine

VOL. XXVI JANUARY, 1959 No. 1

Editorial

The Interplay of Heredity and Environment in the Regulation of Circulating Lipids and in Atherogenesis

ATHEROSCLEROSIS is no longer generally regarded as a degenerative phenomenon associated with aging, and therefore inevitable and irrevocable, but rather as a pathological disorder which is apt to become manifest in later life. There is no agreement, however, as to the pathogenesis of this disorder, and in particular whether it is due to genetic or environmental factors. The view to be discussed here is that the regulation of abnormal levels of circulating lipids, indeed of normal levels, and the consequent predisposition to atherosclerosis is probably determined by genetic factors, which are subject to modification by many external influences.

Of the lipids implicated in atherogenesis, the role of serum cholesterol has been most extensively studied because of the early discovery of cholesterol in atheroma, the frequent occurrence of atherosclerosis in clinical conditions associated with an elevated serum cholesterol, and the experimental production of atherosclerosis by feeding cholesterol to susceptible animals. In considering the etiologic significance of hypercholesteremia, however, the term should first be defined.

In Bloor's original publication 220 mg. per cent was stated to be the upper limit of normal [*1*]. Peters and Man considered 194 mg. per cent ± 36 mg. per cent to be the normal average [*2*]. The upper limit of normality has been taken to be three times the standard deviation above the mean normal, or approximately 300 mg. per cent [*3*]. This level has been selected by many as indicative of hypercholesteremia, whereas others consider 280 mg. per cent and above to be abnormal [*4–6*]. Actually, because of the well established variability of the serum cholesterol level with sex, age and the population under study [*7*], and the variation in results by different methods and indeed by the same method in different laboratories, any definition of hypercholesteremia must be quite arbitrary.

Interest in the lipids related to atherogenesis has been extended in recent years to lipids other than cholesterol—the phospholipids, triglycerides and total lipids. Serum phospholipid levels closely parallel those of serum cholesterol and, at least in our population, appear to be age- and sex-dependent variables [*8*], as will be discussed later.

The definition of hyperlipemia may be based on the gross lactescent appearance of the serum of fasting patients or on the chemically determined elevation of serum triglycerides and/or total lipids. Although the impression prevails that this serum lipid fraction is age- and sex-dependent, no adequate studies are available at present. The designation of "chemical" hyperlipemia (in the fasting state) may be based on

lipid levels above 1200 mg. per cent, whereas gross hyperlipemia (lactescence) occurs at total serum lipid levels of 1,500 to 2,000 mg. per cent or more.

It is apparent that a more precise definition of normal and elevated lipid levels in the serum is needed. We have recently used the upper 5 per cent levels of each five-year age group, in both sexes, as an arbitrary cutting-off point between normo- and hypercholesteremia, and between normo- and hyperphospholipemia [*8,9*] but further study will be required to establish the possible clinical significance of such a demarcation. In considering the difficulties in any such endeavor one should remember that the levels of serum cholesterol and phospholipid are distributed in the populations studied as continuous variables. In this respect the concept of hypercholesteremia and hyperphospholipemia in its present form resembles that recently discussed by Pickering in relation to essential hypertension [*10*].

HEREDITARY FACTORS

Xanthomatosis and Idiopathic Hypercholesteremia. Premature or accelerated atherosclerosis occurs frequently in three clinical conditions, diabetes, hypertension and familial hypercholesteremia, the hereditary character of which are well established [*11*]. That xanthomatosis is hereditary and often associated with coronary artery disease has long been recognized [*12*]. Only recently, however, has it been shown that the elevation of circulating lipids, especially of cholesterol, is more frequently a feature of the disorder than the deposition of lipid (cholesterol plaques) in the arteries, skin or tendons [*4–6*]. In a study involving thirty-five probands with xanthomatosis and their close blood relatives (a total of 201 persons), hypercholesteremia was present in 69 per cent, coronary artery disease in 40 per cent, xanthelasma of the eyelids in 30 per cent, corneal arcus in 18 per cent and tuberous or tendinous xanthoma in only 12.5 per cent [*6*].

The genetic mechanisms of xanthomatosis have been extensively studied but opinion as to the mode of transmission varies considerably [*13–17*]. Those who used the cutaneous lesions as a criterion of the trait considered it to be recessive [*17*]. This mode of transmission is unlikely, however, because of absence of a significant number of consanguineous marriages among the parents of the affected persons [*6*]. Studies based on elevated serum cholesterol levels as a criterion of the trait have led to the now more widely held concept that this syndrome is inherited as an incomplete dominant [*6,18–23*].

Some believe that hypercholesteremia with or without atherosclerosis but without xanthomatosis represents the heterozygous state for the gene; in the homozygous state the gene leads to considerably higher serum cholesterol levels and finally to xanthomatosis and frequent atherosclerosis [*6,18,19,24*]. Other investigators, however, believe that all these manifestations may result from the heterozygous status of the same gene [*21–23*]. There is agreement between both groups that hypercholesteremia and atherosclerosis may occur in the heterozygotes.

Our own recent studies performed in population groups selected at random seem to support the concept that the genetic component involved in the determination of serum lipid levels is a dominant gene with incomplete penetrance [*8,9,25*]. The penetrance of the gene increases with age, perhaps at a younger age in males than in females [*25*]. Although our data do not require a different genetic hypothesis, such as the multiple gene theory, such possibilities are not excluded by the present observations.

Based on the hypothesis that xanthomatosis may represent the extreme expression of an inborn error of lipid metabolism, and that many patients with coronary artery disease might fall into a similar pattern, a group of 122 patients with coronary disease whose average age was forty-five years was studied for the presence of stigmas of hypercholesteremia. Patients known to be members of xanthomatous families were excluded from the study. Of these 122 patients with overt coronary disease at a relatively young age, seventy-one (58 per cent) exhibited hypercholesteremia, twenty-two corneal arcus, twelve xanthelasma and three skin or tendon xanthoma. Families of fifty of these patients were available for study and at least one-third of the siblings were found to be hypercholesteremic [*4,6*].

The clinical data obtained in patients and siblings with coronary artery disease were subjected to a standard genetic test which led to the opinion that the metabolic disturbance of hypercholesteremia was transmitted as a dominant trait [*3*].

A similar study performed in a group of 100 patients in whom evidence of coronary artery disease developed after the age of sixty revealed

a lower incidence of hypercholesteremia and suggested a more indolent metabolic disturbance than that encountered in the younger patients [*26*].

Idiopathic Hyperlipemia. Idiopathic hyperlipemia differs from idiopathic hypercholesteremia in the milky appearance of the serum (lactescence) and in decided elevations of serum triglycerides in the fasting state. Both abnormalities of lipid metabolism may be associated with xanthomatosis, although there are distinct differences in the clinical manifestations [*27*]. In a recently examined group of twenty probands with idiopathic hyperlipemia and their families, xanthoma tuberosum was present in 14 per cent, xanthelasma of the eyelids in 2 per cent and xanthoma tendinosum in 1 per cent. In contrast, in a group of seventy-seven patients with idiopathic hypercholesteremia and their families 2 per cent presented with xanthoma tuberosum, 10 per cent with xanthoma tendinosum and 23 per cent with xanthelasma. These data indicated that xanthomatosis in idiopathic hyperlipemia is usually of the tuberous type, while that of the idiopathic hypercholesteremic is usually of the tendinous variety [*27,28*].

There is a high incidence of coronary artery disease among the index patients with either disorder and among their family members. This observation is in agreement with that of others who demonstrated that patients with idiopathic hyperlipemia have a predisposition to coronary artery disease, not very different from that of idiopathic hypercholesteremia [*27–29*].

Such observations also suggest a possible role for lipids other than cholesterol in the genesis of atherosclerosis, especially since a suddenly induced increase in serum triglycerides in the experimental animal is followed by a secondary elevation of serum cholesterol and phospholipid. Recent studies indicate that abnormal circulating levels of unesterified fatty acids and triglycerides, as well as abnormalities in their transport and metabolism, may in themselves be important factors in atherogenesis [*30–33*].

Investigation of families of probands with idiopathic hyperlipemia revealed that both errors, i.e., idiopathic hyperlipemia and idiopathic hypercholesteremia, may occur in the same family. Study of such families suggests that both disorders are closely related, at least genetically. One may, then, perhaps assume that idiopathic hyperlipemia also is transmitted as a dominant trait with incomplete penetrance [*27*].

Circulating Lipid Levels in Various Populations. In addition to studies of single or multiple families with xanthomatosis and/or coronary artery disease, numerous attempts have been made to determine the serum cholesterol levels and the incidence of hypercholesteremia in various population groups [*34*]. More recently, Keys and co-workers carried out extensive studies on men of various ages in the United States, England, Spain, Sweden, Italy, Japan and Finland [*35,36*]. Epstein, Boas and coworkers [*37*] included serum cholesterol studies in their observations on manifest atherosclerosis in a population in New York City which consisted mainly of Jews and Italians. Oliver and Boyd performed plasma cholesterol determinations in men and women in Scotland [*38*]; Walker and Arvidson studied cholesterol levels in the South African Bantu [*39*]. A study of the available data reveals marked differences in the circulating cholesterol levels among the various population groups. Whether these differences are caused by genetic factors, by diet or other environmental influences, or by widespread pathological conditions, malnutrition, parasitic infestations or hepatic disease, cannot be decided at present [*25*]. It may be concluded that each survey of circulating lipid levels is valid for the specific population only and that great caution must be exercised in comparing populations living under different geographic, cultural and socioeconomic conditions.

Another survey was concerned with a study of 250 men and 250 women, consecutive admissions to medical wards of a large general hospital in New York City [*40*]. The data on serum cholesterol were evaluated by the standards of Keys and associates in which variation with age was considered. The frequency of idiopathic hypercholesteremia for the total sample was 12.0 per cent and the frequency among the siblings and children of the hypercholesteremic probands was 36 and 34 per cent, respectively. These frequencies were, then, significantly greater than those found in the index patients. Hypercholesteremia was significantly more frequent among the Jewish probands than among the non-Jewish (21 per cent versus 9 per cent). The frequency among siblings and children was not different, however, when Jewish hypercholesteremic probands and families were compared with non-Jewish subjects. This find-

ing would suggest that environmental factors are of less decisive importance than hereditary factors in determining the presence of idiopathic hypercholesteremia [9].

A study of individuals selected at random included 201 "healthy" families, comprising 775 persons, and an additional 461 persons unrelated to the others, a total of 1,236 individuals in New York City [9]. In males, the mean serum cholesterol level remained essentially constant to age twenty. From then through age thirty-two, there was a significant increase averaging 5.6 mg. per 100 cc. per year. Thereafter, until age sixty, there was no further change. In females, no significant change in the average serum cholesterol level was observed from age two through thirty-two. From age thirty-three through fifty-eight a significant rate of increase of 3.0 mg. per 100 cc. per year occurred. The period of marked physiologic increase of serum cholesterol level with age started in males about thirteen years earlier than in females. The annual increments were also greater in the male. The difference in age trends between men and women may have some bearing on the well known preponderance of coronary artery disease in the younger age groups in males [9]. The difference in the effect of male and female sex hormones on circulating lipids and lipoproteins is in good agreement with this observation [41,42].

A correlated study was concerned with the levels of serum phospholipids in the same healthy population [8]. This study supplied much needed information on possible relationships between age, sex and circulating phospholipids. It included 1,067 normal persons. Among these were 156 families comprising 580 persons, and an additional 487 unrelated individuals. Analysis of the data revealed that serum phospholipid levels varied with age and sex in a manner similar to that of the serum cholesterol. In males there is a sharp increase from age twenty to thirty-two, averaging 5.0 mg. per 100 cc. per year. In females an increase begins at age thirty-two until age sixty, and averages 2.1 mg. per 100 cc. per year. There was a very high correlation between serum cholesterol and phospholipid levels in both males and females. For each rise of 1.0 mg. of serum cholesterol there was a rise of 0.72 mg. serum phospholipid in males and 0.68 mg. in females. In the light of the high correlation between serum cholesterol level and serum phospholipid level, the genetic analyses of these two variables paralleled each other closely. Continuous distribution of the levels is evident for both lipid fractions (continuous variables).

Factors Regulating Levels of Circulating Lipids. If hereditary factors were of primary importance in determining circulating lipid levels in healthy persons, one would expect no correlation between the blood lipid levels of fathers and mothers (since there is no blood relationship), whereas the levels of each parent and the children as well as those of the siblings should be positively correlated. Alternately, if environmental factors are of primary importance the blood lipid levels of the parents should be correlated with each other as well as with those of their children, since they all share the same environment.

In such a study [8,9], the computed correlation coefficients, corrected for age and sex variation, revealed that the correlation coefficients between the parents in respect to both serum cholesterol and phospholipid were essentially zero. Those between father and child, mother and child, and between siblings, on the other hand, were significant [8,9]. These data are consistent with the concept that there is an important genetic component in the regulation of serum cholesterol and serum phospholipid levels. No indication was obtained that the gene or genes determining these levels were sex-linked.

Recent studies in monozygotic and dizygotic adult twins in good health again demonstrated the importance of both genetic and environmental factors in the regulation of serum lipid levels. Since genetic differences are a known quantity in twins, these results are of particular interest. They suggest that genetic factors are not equally important in all environments, and that the environmental tolerances of one population are not the same as those of another [43].

ENVIRONMENTAL FACTORS

The genetic determination of circulating lipids does not, of course, exclude the contribution of environmental factors to the regulation of these levels. Extensive clinical, epidemiological and experimental work indicates that dietary factors, especially fat and protein, affect the serum cholesterol level and presumably influence the course of arterial disease [35,44–46]. The ratio of saturated to unsaturated fats in the diet is known to be a factor in the regulation of circulating cholesterol and lipoprotein levels [35,45,47]. Less is known, but much has been

hypothesized, regarding such factors as ethnic origin, occupation, stress, exercise and tobacco and alcohol consumption [*35,47,48*]. It is possible that the endocrine system, which is known to exert a strong influence on lipid metabolism [*42*], represents the intermediary mechanism between genetically-determined enzymatic chain reactions and environmental influences.

The individual susceptibility to coronary artery disease in populations with a low incidence of this disorder, perhaps related to low intake of dietary fat and protein, is probably based on a genetic predisposition. Contrariwise, in populations with a high incidence of coronary artery disease, perhaps related to a high intake of fat and protein or to other environmental influences, failure of the disease to develop in many persons in the same environment presumably is ascribable to the absence of a predisposing genetic factor, as in other inherited metabolic disorders such as diabetes and gout. Strong environmental influences thus can modify and even nullify the genetic predisposition. This situation may prevail among the Japanese [*49*], the rural Bantus [*39*], the Yemenites [*46*], the rural Guatemalans [*50*] and other populations.

It appears, then, that the interplay between genetic and environmental factors determines the susceptibility to atherosclerosis. This pertains to population groups and to individuals within population groups [*43*].

Although our knowledge in this field has increased greatly in recent years, many questions remain unanswered [*51*]. Little is known about the mechanisms involved in idiopathic hypercholesteremia and hyperlipemia, about changes in the tissues, and the enzymatic disturbances at the cellular level.

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Clinical Studies

Familial Hypercholesteremic Xanthomatosis: A Preliminary Report*

I. Clinical, Electrocardiographic and Laboratory Considerations

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THE syndrome of xanthomatosis (cutaneous and tendinous) associated with essential or primary hypercholesteremia has long been recognized. The familial nature of this syndrome has been amply attested [*1–9*] although there continues to be a lack of unanimity concerning the exact mode of transmission. It is known that, in many instances, hypercholesteremia presents itself as the only indication of the abnormal trait. This is known as the "forme-fruste" of the condition.

From the clinical standpoint, the chief interest of this inborn error of metabolism lies in its frequent association with coronary artery disease. This feature has been largely responsible for what might be termed the chemical approach to the problem of atherosclerosis, which has led to the current intensive investigation into virtually all phases of lipid metabolism.

This study was undertaken in an attempt to correlate the lipid and lipoprotein determinations with the clinical and electrocardiographic findings in two families afflicted with the hypercholesteremic trait. The normocholesteremic members of these families constituted a "built-in" control group. It is my intention to conduct periodic follow-up studies in order to chart the natural history of this unique although by no means rare anomaly. Another motivation was the anticipation that this type of study might add to our knowledge of the natural fluctuations in the serum cholesterol and other lipids of normal and hypercholesteremic persons, as determined at intervals over long periods of time.

MATERIAL AND METHODS

One hundred and three persons have been studied, ninety-nine in one family (to be designated kindred I) and four in the other (kindred II). Fourteen of the patients were the spouses of members of kindred I. These patients were included in the study because the data derived therefrom were essential in analysing the mode of inheritance of this disorder. Wherever possible, patients were admitted to Lancaster Hospital for the investigation, but in the majority of cases it was necessary to carry out the examinations in their homes, some being as far away as 350 miles. All but four patients were seen and examined by me. Of these, one had been thoroughly examined elsewhere and the complete case report, electrocardiogram and a sample of blood were forwarded by his physician. In another case, a fifteen year old normocholesteremic girl, blood samples were sent to our laboratory for serum lipid determinations. I have been assured by the parents that a recent physical examination had uncovered no evidence of heart disease or xanthomatosis. Only the serum lipid data on this case (27C) are shown, however, as the clinical information came from a non-medical source. Two patients were fully examined by Capt. John Burris, R.C.A.M.C., a former associate. The clinical data on these cases, and specimens of blood serum, were forwarded from Whitehorse, Yukon Territory.

Most patients had fairly complete examinations, consisting of functional inquiry, past medical history, family history with particular reference to cardiac

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TABLE I
DISTRIBUTION OF CASES

Clinical Data	Hypercholesteremic (over 300 mg. %)	"Borderline" (250 to 300 mg. %)	Normocholesteremic (140 to 250 mg. %)
Complete examination	36	17	17
Incomplete examination	5	8	19
No examination (serum lipids only)	..	..	1
Total	41	25	37

and vascular disease, and routine physical examination. In some the examination was incomplete, consisting of an inquiry concerning cardiovascular, cerebrovascular and peripheral vascular symptoms, coupled with a search for arcus senilis/juvenilis and tendon and cutaneous xanthomas, including xanthelasma. Some members of the two families live in areas so far removed from this center that it has not yet been possible to examine them.

In Table I is shown the distribution of cases according to the extent of the clinical examination and the serum cholesterol zones.

In those patients who were admitted to hospital and in some who were examined in the out-patient department, the investigation included roentgenograms of the chest, cholecystograms and x-ray films of the pelvis and legs for evidence of arterial calcification. Liver function tests, serum protein and blood sugar determinations were carried out in most of these cases. Basal metabolic rates were determined when they were considered to be indicated. With only six exceptions, all blood samples were taken in the fasting state. Only the serum cholesterol and lipid phosphorus estimations were carried out on the non-fasting serums. We have ascertained, as have others, that these determinations are not significantly influenced by the postprandial state.

Early in the course of the study, it was established in our laboratory that allowing the serum to stand for several days, even at room temperature, did not affect the total serum cholesterol levels to any significant extent. A similar observation has been reported by Sperry and Webb [*10*]. It has also been shown [*11*] that serum can be stored at 0° to 5°C. for up to twenty-eight days without altering the lipoprotein pattern or content to any significant degree. These findings were of considerable importance since, in many instances, blood samples were drawn in the patients' homes, and were then refrigerated until they were transported (by automobile) to our laboratory. The blood was collected in sterile tubes in all but a few instances. The period of non-refrigeration was usually no longer than two to three hours, but in a few cases the tubes of blood were in transit up to seven days before reaching the laboratory. Since these were shipped by air during our northern winter, it may be assumed that shipping temperatures were low, but inconstant.

All serum determinations were made by one thoroughly trained technician. All the cholesterol, and some of the other lipid analyses were carried out in duplicate. The lipid determinations included the following, although not all of these were carried out in every case:

1. Cholesterol: the Bloor method [*12*], slightly modified as outlined in Evelyn's Handbook on Photoelectric Colorimetry, was used throughout. A few words concerning the accuracy of this method are, I believe, germane to this presentation, since it has been stated not infrequently that this method is less accurate than that of Schoenheimer-Sperry. In a series of seventy-five consecutive Bloor determinations of the serum cholesterol, there was a discrepancy between duplicate readings of less than 4 per cent in all the serums; in the majority of these the discrepancy was 2 per cent or less. In a somewhat smaller number of tests using the Schoenheimer-Sperry method, the degree of reproducibility was no better. Moreover, the latter test is considerably more time-consuming and laborious than is the Bloor method. Both methods were employed by Keys et al. [*13*] in a large survey of the serum cholesterol of 2,056 persons. Five thousand determinations were done and the writers found such a small disparity between the two methods that they did not deem it necessary to make any distinction between the results obtained by each. More recently, Thomas and Eisenberg [*14*], using a modification of the Bloor method, reported a high degree of duplicate reproducibility and concluded that the method was eminently satisfactory for research purposes.

2. Cholesterol esters: The method employed was that of Bloor and Knudson [*15*]. This determination, of course, could be made on fresh serums only, a consideration which excluded all the blood samples collected in the patients' homes. However, in all cases in which the test was performed the cholesterol esters were found to be normal. This is in accord with the findings of others who have investigated this condition.

3. Lipid phosphorus: the method of Youngsburg and Youngsburg [*16*] was used, modified in that phosphorus was measured by the Gomori method instead of that of Fiske and Subbarow.

4. Total serum lipids: these were estimated by the method outlined in the U. S. Naval Medical School manual "Physiological Chemistry" [*17*].

Ultracentrifugal lipoprotein studies: Aliquots of serum were sent, under sterile conditions, to the Ultracentrifuge Laboratory, McGill University, where ultracentrifugal analyses of lipoproteins were carried out. In three cases (1A, 3A, 3B) three consecutive specimens of serum were pooled in order to obtain a large enough sample for lipoprotein determination.

Electrophoretic studies: For the past year we have been making electrophoretic analyses of proteins and lipoproteins on all serums received. Because of the relatively small number of cases so studied these results will not be included in this report.

RESULTS

Lipids and Lipoproteins (*Tables* II, III *and* IV). In those cases in which more than one set of lipid and lipoprotein determinations were carried out, no more than the first four determinations are shown.

Serum Cholesterol. The normal range for total serum cholesterol by the Bloor method is said to be from 140 or 150 to 250 mg. per 100 ml., although Wilkinson et al. [*3*] consider 280 mg. per cent to represent the upper limits of normal. Because of a rather universal uncertainty regarding the significance of cholesterol values which fall in the "no-man's land" between 250 and 300 mg. per 100 ml., it seemed expedient to designate cholesterol results in this zone as "borderline"; those below 250 mg. were classed as normocholesteremic and those above 300 mg., hypercholesteremic.

Various workers [*13,18*] have observed that the serum cholesterol tends to rise with increasing age until middle life in healthy males, and somewhat later in healthy females, after which a modest downward trend occurs. Sperry and Webb [*10*] were of the opinion that, while some persons did show an increase in the serum cholesterol concentration with the passing of time, others did not show this type of change during a thirteen to fifteen year follow-up period. They concluded that an increase in the serum cholesterol is "not an obligatory concomitant of aging." Moreover Keys et al. [*13*] have tempered their own findings (which strongly suggested an age-cholesterol relationship) by the statement that "it is entirely possible that persons who are individually characterized by having relatively low cholesterol concentrations tend to survive longer than their fellows who maintain higher levels."

Notwithstanding this cautionary observation, it seems to be generally acknowledged that an age relationship exists in some, perhaps most, people. Pending the amassing of more decisive data one is justified in assuming that a borderline serum cholesterol in a middle-aged or elderly person may actually be normal, while the same levels in a young person might well prove to be hypercholesteremic.

Hypercholesteremic Group (*Table* II). There were forty-one persons in this group, thirty-eight in kindred I and three in kindred II. Interestingly enough, two of these (Cases 31A and 33A) were the spouses of hypercholesteremic members of kindred I and a third (Case 40A) was married to another member of this kindred (Case 19B) in whom the cholesterol level was in the borderline zone. This unexpectedly high incidence among the spouses tends to lend support to the observation of various authorities that hypercholesteremia *per se* is not at all uncommon. The genetic implications of such matings were of particular interest and will be reported in a later paper.

All three hypercholesteremic members of kindred II (Cases 20A, 21A and 22A), were females. In kindred I there were twenty males and eighteen females. The youngest patient was three years old, the oldest sixty-nine. The serum cholesterol levels ranged from 314 mg. to 954 mg. per 100 ml.

The lipid phosphorus was elevated more or less in proportion to the cholesterol levels. One exception to this observation was the finding of a lipid phosphorus of 12.8 mg. in Case 1A at a time when the cholesterol was 473 mg. Since an earlier reading of 15.3 mg. was obtained with a cholesterol of 495 mg., it seems possible that the discrepancy was due to a technical error.

The total lipids also were found to be elevated somewhat in proportion to the serum cholesterol. It is often stated that the total serum lipid and neutral fat concentrations are increased only slightly, if at all, in primary hypercholesteremia. However, some of these publications present laboratory data indicating that more marked elevations of the total lipids (to as high as two to three times normal) may actually occur [*1,7*]. Such elevations are, of course, not of the magnitude commonly seen in idiopathic hyperlipemia,

from which familial hypercholesteremia must be distinguished. Although some of the patients with hypercholesteremia in this study were found to have rather high total lipid values, idiopathic hyperlipemia was ruled out on the basis of a clear serum and the absence of splenomegaly, eruptive xanthomas and abdominal pain.

Three patients had diseases which are, in themselves, often associated with hypercholesteremia. One patient (Case 19A) was found to have myxedema in 1948, at which time her serum cholesterol (determined by another laboratory) was 350 mg. per cent. Despite excellent clinical control with desiccated thyroid therapy, the serum cholesterol rose in the intervening seven years to 820 mg. per cent. This woman has two hypercholesteremic children, so there can be no doubt of the coexistence of familial hypercholesteremia with hypothyroidism, the former being the major factor in the persistence and actual progression of the hypercholesteremia.

Case 22A is of particular interest. This patient has been proved, both on clinical grounds and by repeated biopsies of the liver, to be suffering from pericholangiolitic cirrhosis as well as familial hypercholesteremic xanthomatosis, a combination of diseases which has not hitherto been reported. The latter diagnosis rests on (1) clinical evidence of the appearance of xanthomatous lesions before the onset of the liver disease and (2) a strong family history of hypercholesteremic xanthomatosis (Cases 20A and 21A are sisters of this patient and the mother, who died at forty-six of coronary occlusion, was known to have tendon xanthomas).

Another patient (Case 24A) has mild diabetes mellitus, controlled by diet alone. Diabetes is often proclaimed to be a common cause of hypercholesteremia. In actual fact, however, the lipid disorder associated with diabetes is a hyperlipemia, i.e., an elevation of the serum neutral fat concentration, as a concomitant of which there is a rise, usually of modest proportions, in the serum cholesterol. These changes develop in diabetic persons who are under poor control, many of whom have acidosis when the cholesterol is determined. I do not believe that hypercholesteremia in well controlled diabetic persons can be ascribed to the diabetes *per se*, except in the presence of the so-called Kimmelstiel-Wilson syndrome in which the serum cholesterol is not infrequently elevated. As in the preceding case (Case 22A) there was a high incidence of hypercholesteremia in the immediate members of this patient's family.

A fourth patient (Case 39A) was found to be hypercholesteremic despite the fact that neither of her parents nor any of the siblings was hypercholesteremic (two of the latter had "borderline" serum cholesterol levels). In this case, blood was obtained two weeks postpartum and, until further determinations are carried out, I cannot be certain that the elevation of serum cholesterol was not due to hormonal factors associated with the recent pregnancy, although this does not seem likely since it has been shown that hypercholesteremia of this etiology subsides after the thirtieth week of pregnancy [*19*].

Age Trends. The mean serum cholesterol level in the decade 0 to 10 years (five cases) was 376.6 mg. per cent; in the decade eleven to twenty years (three cases), 351.3 mg. per cent; in the decade twenty-one to thirty years (four cases), 435.7 mg. per cent; thirty-one to forty years (ten cases), 548.7 mg. per cent; forty-one to fifty years (seven cases), 424.4 mg. per cent; fifty-one to sixty years (five cases), 410 mg. per cent; and sixty-one to seventy years (seven cases), 530.1 mg. per cent. These values were derived from the first cholesterol determination in each case. The number of cases in each age group is much too small to permit any valid conclusions regarding variations of the serum cholesterol in relation to age.

"Biologic Variation" of the Serum Cholesterol. Not enough patients have had repeated serum lipid determinations to allow one to comment definitively on the individual variability of the serum cholesterol. However, the findings do permit the tentative conclusion that some hypercholesteremic subjects show a rather marked constancy and others a considerable degree of lability with respect to their serum cholesterol levels. Frequent determinations over extended periods of time are necessary to determine whether the subject is stable or labile in this regard. One patient (Case 3A), who has had numerous lipid analyses over a three year period, has shown remarkably constant cholesterol levels during this time, uninfluenced by rigorous dietary restrictions of fat. Administration of sitosterol did, however, produce significant depressions of the serum cholesterol, and a particularly pronounced drop was observed to occur as a result, apparently, of the "stress" produced by second degree burns. Several other patients have demonstrated a similar constancy

TABLE II

SERUM LIPIDS AND LIPOPROTEINS IN HYPERCHOLESTEREMIC SUBJECTS

Case No.	Patient's Age (yr.) and Sex	Date (day, mo. and yr.)	Total Cholesterol (150–250 mg. %)	Lipid Phosphorus (9–12 mg. %)	Total Lipids (400–800 mg. %)	Lipoproteins, S_f			
						0–12	12–20	20–100	100–400
1A	58, M	20-9-54	495	15.3	1715	....	...	...	...
		27-9-54	436	....	....	....	...	...	...
		23-11-54	488	....	....	....	...	...	...
		24-11-54	473	12.8	....	675	180	497	86
2A	55, M	14-12-54	452	13.8	1840	783	166	284	87
		16-12-54	430	13.9	1466	....	...	...	...
		20-12-54	450	14.2	....	....	...	...	...
		21-12-54	448	13.7	....	....	...	...	...
3A	41, M	20-8-54	500	15.1	....	....	...	...	...
		23-11-54	480	14.8	1640	....	...	...	...
		24-11-54	468	15.3	....	....	...	...	...
		26-11-54	492	14.9	....	938	235	316	76
4A	43, F	24-8-54	517	....	....	....	...	...	...
		8-1-55	506	16.8	....	....	...	...	...
		15-1-55	515	17.6	1720	856	251	146	40
		22-1-55	510	17.8	1990	....	...	...	...
5A	68, F	15-8-55	676	23.0	1860	896	199	329	23
		30-8-55	742	23.9	2080	789	205	486	154
6A	42, M	20-9-54	380	13.1	1879	....	...	...	...
		27-9-54	392	13.8	....	....	...	...	...
		18-1-55	490	16.9	....	802	138	98	17
		19-1-55	458	15.1	1300	729	123	125	23
7A	61, F	25-1-55	528	18.1	1425	875	217	200	58
		28-9-55	616	20.3	....	....	...	...	...
8A	35, M	25-1-55	632	19.7	1820	1013	326	267	52
		3-3-55	616	19.8	1410	1069	196	101	23
		22-9-55	684	20.0	1600	1003	154	92	0
		29-9-55	608	18.7	....	....	...	...	...
9A	68, M	14-6-55	440	15.6	1220	686	107	71	24
10A	32, F	31-5-55	424	19.1	1175	671	80	83	6
		19-11-57	494	....	....	....	...	...	...
11A	8, M	8-6-55	396	15.2	1110	589	84	8	0
		19-11-57	420	14.5	....	....	...	...	...
12A	19, M	26-1-55	380	13.8	1200	....	...	...	...
		20-8-56	384	14.1	1035	517	77	57	0
13A	46, M	25-1-55	600	19.1	1800	951	218	323	52
		12-10-57	480	14.7	1360	....	...	...	...
14A	58, M	26-1-55	334	14.4	1140	615	138	171	34
15A	27, F	16-8-55	417	16.1	1040	692	84	39	6
		8-8-57	480	16.3	1215	....	...	...	...
16A	40, M	17-4-55	568	19.1	1550	....	...	...	...
		13-10-56	640	19.1	1420	1019	148	53	0
17A	28, M	22-8-55	454	16.6	....	....	...	...	...
		29-8-55	465	15.6	1275	718	101	88	30
18A	32, M	26-1-55	438	15.6	1100	....	...	...	...

TABLE II (*Continued*)

SERUM LIPIDS AND LIPOPROTEINS IN HYPERCHOLESTEREMIC SUBJECTS

Case No.	Patient's Age (yr.) and Sex	Date (day, mo. and yr.)	Total Cholesterol (150–250 mg. %)	Lipid Phosphorus (9–12 mg. %)	Total Lipids (400–800 mg. %)	Lipoproteins, S_f			
						0–12	12–20	20–100	100–400
19A	63, F	10-10-55	820	25.6	2300	1474	255	122	0
		24-5-56	712	25.0	1775	984	221	99	0
		9-10-57	676	21.9	....	....	...	...	...
20A	30, F	7-3-55	494	15.3	1720	904	123	52	0
		15-2-56	464	14.8	1040	1028	104	27	0
21A	34, F	25-7-55	652	19.7	1590	1038	148	112	36
		5-7-56	606	20.3	1595	974	172	124	12
22A	37, F	7-3-55	860	31.3	2230	1277	377	185	11
		14-3-55	954	33.7	2210	1202	486	200	11
		31-5-55	880	34.2	2055	1446	290	134	0
		25-7-55	772	25.6	2050	1072	282	226	23
23A	35, M	30-11-55	510	18.4	1692	592	154	431	60
24A	64, F	1-12-55	544	19.1	1683	1119	114	23	0
		24-5-56	626	19.7	1570	1152	93	0	0
25A	37, M	29-11-55	452*	18.1	....	....	...	...	...
			570	....	....	....	...	...	...
26A	44, M	1-12-55	314*	12.2	....	....	...	...	...
		14-12-55	362	13.4	1080	612	82	50	6
27A	11, M	14-12-55	340	13.4	....	523	73	20	6
28A	9, F	25-7-56	325	....	....	....	...	...	...
		13-10-56	349	12.2	820	....	...	...	...
29A	55, M	15-10-56	426	15.6	1040	....	...	...	...
30A	3, F	5-10-56	393	14.7	....	....	...	...	...
31A†	58, F	15-10-56	343	14.3	....	....	...	...	...
		22-11-56	363	15.2	1030	540	101	83	24
32A	11, M	15-10-56	334	13.4	950	589	127	83	6
33A†	69, F	22-11-56	343	15.3	1030	516	124	287	71
		31-12-57	312	12.8	....	....	...	...	...
34A	48, F	29-1-57	324	13.3	1085	402	92	124	66
		27-2-57	271	12.5	860	339	91	151	29
		25-3-57	261	11.2	....	....	...	...	...
35A	35, M	29-1-57	375	14.1	1235	518	129	275	83
36A	28, F	23-8-57	378	17.5	1380	....	...	...	...
37A	34, F	23-8-57	556	19.1	1505	....	...	...	...
38A	9, M	10-10-57	329	....	....	....	...	...	...
39A	45, F	10-10-57	336	15.9	....	....	...	...	...
40A†	68, F	27-2-57	360	13.7	1110	509	142	163	18
41A	6, F	19-11-57	440	....	....	....	...	...	...

NOTE: Figures in parentheses in column headings represent normal values.
* Non-fasting serum.
† Spouse.

TABLE III
SERUM LIPIDS AND LIPOPROTEINS IN "BORDERLINE" SUBJECTS

Case No.	Patient's Age (yr.) and Sex	Date (day, mo. and yr.)	Total Cholesterol (150–250 mg. %)	Lipid Phosphorus (9–12 mg. %)	Total Lipids (400–800 mg. %)	Lipoproteins, S_f			
						0–12	12–20	20–100	100–400
1B	73, F	22-9-55	276	12.5	750	348	58	161	56
2B	31, F	27-9-55	271	11.9	770	359	65	103	18
3B	65, M	23-11-54	258	9.2	1120	...	..	...	..
		24-11-54	258	9.8	....	...	..	...	..
		25-11-54	249	9.8	....	422	67	110	31
		26-11-54	256	9.7	....	...	..	...	..
4B	35, F	27-9-55	267	11.7	730	363	77	26	6
5B	67, F	10-10-55	280	12.8	900	...	..	...	..
6B	42, M	17-4-55	266	11.9	720	...	..	...	..
		14-10-56	297	12.2	690	415	73	19	0
		24-8-57	237	10.3	590	...	..	...	..
7B	28, F	25-1-55	299	14.0	1120	476	32	116	17
		23-8-56	292	11.9	740	432	57	66	18
		9-10-57	256	11.9	....	...	..	...	..
		22-10-57	263	11.9	800	...	..	...	..
8B	38, M	30-5-55	264	12.0	725	428	17	88	11
9B	51, M	22-9-55	253	11.3	850	346	78	146	30
		28-5-57	239	12.0	965	...	..	...	..
		29-5-57	227	10.9	860	...	..	...	..
10B	68, F	29-5-55	225	11.3	588	314	23	39	0
		28-9-55	285	11.4	....	...	..	...	..
		3-10-57	249	12.2	....	...	..	...	...
11B*	49, F	22-9-55	296	12.5	800	331	33	30	6
		1-12-55	236	12.1	....	...	..	...	..
		1-10-57	251	11.9	810	...	..	...	..
		2-10-57	260	....	....	...	..	...	..
12B	22, M	14-12-55	254	12.7	835	343	13	6	12
		4-1-56	227	10.9	....	...	..	...	..
13B	35, M	14-12-55	270	12.5	....	410	58	88	6
14B*	38, F	22-11-56	277	12.5	880	431	68	85	30
15B	42, M	28-11-56	282	13.3	1060	280	68	155	71
		6-2-57	249	11.4	730	...	..	...	..
		7-2-57	248	10.3	....	...	..	...	..
16B*	60, F	10-1-57	257	11.4	725	324	66	48	0
17B	25, M	13-1-55	214	8.9	....	...	..	...	..
		8-8-57	257	10.8	740	...	..	...	..
		20-12-57	214	9.4	585	...	..	...	..
18B	63, M	7-8-57	250	10.6	710	...	..	...	..

TABLE III (*Continued*)
SERUM LIPIDS AND LIPOPROTEINS IN "BORDERLINE" SUBJECTS

Case No.	Patient's Age (yr.) and Sex	Date (day, mo. and yr.)	Total Cholesterol (150–250 mg. %)	Lipid Phosphorus (9–12 mg. %)	Total Lipids (400–800 mg. %)	Lipoproteins, S_f			
						0–12	12–20	20–100	100–400
19B	67, M	23-8-55	128	....	....	...	..	...	..
		15-9-55	215	....	....	...	..	...	..
		14-10-55	227	12.5	667	294	36	38	0
		19-3-57	268	12.5	745	387	53	105	28
20B*	66, M	9-10-57	262	11.9	....	...	..	...	..
21B	37, F	10-10-57	256	12.2	....	...	..	...	..
22B	F	22-10-57	260	13.1	855	...	..	...	..
		23-10-57	218	....	....	...	..	...	
23B	43, M	3-10-57	250	12.5	820	...	..	...	..
		4-10-57	236	....	....	...	..	...	...
24B	40, F	28-10-57	261	12.5	850	...	..	...	..
25B*	35, M	19-11-57	265	10.3	....	...	..	...	..

NOTE: Figures in parentheses in column headings represent normal values.
* Spouse.

of the cholesterol pattern. Others (Cases 6A, 7A, 19A and 22A) have shown wide swings in their cholesterol levels.

Only one patient (Case 34A) showed a drop in the cholesterol level to values below 300 mg. per cent. This was probably attributable to dietary factors, since the fall was observed to occur several months after the institution of a low calorie, moderately low fat diet.

The problem of the natural or "biologic" variation in the serum cholesterol levels of subjects as determined at different times has been dealt with by various authors. Sperry [*20*] contended that in healthy persons the serum cholesterol is maintained at relatively constant levels, with minor deviations only, perhaps throughout life. On the other hand, Thomas and Eisenberg [*14*] in a recent study of healthy medical students, concluded "that biologic variability from causes other than aging is a conspicuous characteristic of the cholesterol levels of many young adults." Wilkinson [*21*], has observed wide fluctuations of the serum cholesterol in some of his patients during lengthy control periods of two years or more. He cites graphically several cases in which the cholesterol levels showed swings from well within normal to markedly hypercholesteremic levels (in one case from approximately 235 mg. per cent to 430 mg. per cent). Based on their observation of cholesterol fluctuations averaging 50 mg. per cent, Welin and Crame [*22*] contend that one should not rule out a diagnosis of hypercholesteremia on the basis of a single cholesterol determination, since subsequent readings might prove to be hypercholesteremic. With the exception of the one case, mentioned earlier, in which the serum cholesterol dropped from hypercholesteremic to "borderline" levels (probably owing to dietary factors), I have not observed such wide fluctuations in this study; i.e., no other hypercholesteremic patient showed a drop in the cholesterol level to below 300 mg. per cent, nor did any of the normocholesteremic or "borderline" subjects show elevations to over 300 mg. per cent at any time, although in a number of cases cholesterol levels fluctuated between normal and "borderline."

Lipoprotein Analyses in Hypercholesteremic Subjects. The serum lipoproteins were determined by the analytic ultracentrifuge method evolved by Gofman and his co-workers. The reader is referred to the publications of this group [*23–26*] for an outline of the methods and some of the applications of this relatively new approach.

TABLE IV

SERUM LIPIDS AND LIPOPROTEINS IN NORMOCHOLESTEREMIC SUBJECTS

Case No.	Patient's Age (yr.) and Sex	Date (day, mo. and yr.)	Total Cholesterol (150–250 mg. %)	Lipid Phosphorus (9–12 mg. %)	Total Lipids (500–800 mg. %)	Lipoproteins, S_f			
						0–12	12–20	20–100	100–400
1C	35, M	17-4-55 23-8-56	200 193	10.7	815 ...	... 290	.. 47	... 42	.. 6
2C	65, F	15-2-55 29-10-57	219 238	12.5 11.6	... 840	... ...	.. ..	... ...	.. ..
3C	81, F	29-5-55	187	10.6	825	...	..	...	..
4C	43, F	17-6-55	242	12.8	...	...	..	...	..
5C	12, M	4-6-55	232	11.8	540	241	17	21	0
6C	39, F	10-10-55 1-10-57 2-10-57	187 188 189	9.7 10.3	610 680 ...	... ...	.. .. ..	... ...	.. .. ..
7C	33, F	10-10-55 3-1-58	195 196	10.8 9.8	710 660	... ...	.. ..	... ...	.. ..
8C	38, M	10-10-55	195	9.1	600	265	35	6	0
9C	F	5-7-55	227	9.5	...	...	..	...	..
10C	29, F	22-9-55 22-10-57 23-10-57	200 248 205	10.0 11.3	560 905 ...	232	47	24	0 .. ..
11C	40, F	27-9-55 2-11-55	208 218	9.7 9.8	580 510	280 312	24 47	36 24	6 0
12C	47, M	16-6-55	232	11.1	700	348	63	144	69
13C*	35, F	22-9-55 27-9-55	227 237	10.0 10.9	610 ...	256 ...	41 ..	30 ...	0 ..
14C*	39, F	25-7-56	226	10.6	705	288	25	7	0
15C	12, F	25-7-56	198	9.9	690	256	23	18	0
16C*	30, F	25-7-56	240	11.6	760	346	48	64	12
17C	11, F	25-7-56	185	10.3	690	261	0	0	0
18C*	33, F	23-8-56	200	9.8	550	276	0	0	0
19C	9, F	23-8-58	208	10.0	...	276	36	79	6
20C*	28, M	28-8-56	212	10.9	720	286	34	129	84
21C	17, F	15-10-56	183	9.7	...	...	..	...	..
22C	15, M	15-10-56	170	9.1	540	235	33	73	6
23C	8, F	15-10-56	165	9.4	...	247	33	62	0
24C	5, M	15-10-56	175	8.4	...	...	..	...	..

TABLE IV (*Continued*)

SERUM LIPIDS AND LIPOPROTEINS IN NORMOCHOLESTEREMIC SUBJECTS

Case No.	Patient's Age (yr.) and Sex	Date (day, mo. and yr.)	Total Cholesterol (150–250 mg. %)	Lipid Phosphorus (9–12 mg. %)	Total Lipids (400–800 mg. %)	Lipoproteins, S_f			
						0–12	12–20	20–100	100–400
25C	24, F	21-11-56	212	9.7	570	267	46	72	0
26C	13, F	22-11-56	150	9.1	530	213	38	78	12
27C	15, F	21-11-56	174	8.3	695	...	..	...	..
28C	12, F	23-8-57	220†	....	...	...	..	...	..
29C	29, F	7-8-57	186	10.3	...	..	..	...	..
30C	6, M	9-10-57	195	9.4	...	...	..	...	..
31C	9, M	9-10-57	163	8.4	...	...	..	...	..
32C*	51, M	9-10-57	233†	12.0	...	...	..	...	..
33C*	41, F	12-10-57	184†	9.9	...	...	..	...	..
34C	9, F	12-10-57	144†	8.4	...	...	..	...	..
35C*	45, F	12-10-57	183	....	...	...	..	...	..
36C	35, M	28-10-57	224	11.9	...	...	..	...	..
37C*	72, M	29-10-57	240	11.3	...	...	..	...	..

NOTE: Figures in parentheses in column headings represent normal values.
* Spouse.
† Non-fasting serum.

The refinement introduced by deLalla and Gofman, by which such factors as slowing of the macromolecular migration rate with increasing lipoprotein concentration during ultracentrifugation are taken into account, and which have led to the use of the term "standard" as a prefix to the lipoprotein measurements, is not used at the McGill Ultracentrifuge Laboratory. The error entailed in the older method is not believed to be of sufficient magnitude to affect significantly the results obtained.

The results of the lipoprotein analyses are shown in Tables II, III and IV. They are expressed in mg. per 100 ml. Not enough subjects have been studied to enable one to delineate the normal ranges for the various lipoprotein fractions. However, Glazier et al. [*27*] have recorded quite extensive data dealing with the "standard" lipoprotein values in apparently healthy subjects at different age levels. Although, as mentioned before, our results do not take into account the slowing effect of concentration (this effect is corrected for in the designation "standard lipoprotein"), it is believed that these normal values, as determined by Glazier et al., may be applied in a broad sense to the results obtained in this study.

Although not all the lipoprotein analyses have been completed as yet, enough results are at hand to permit certain tentative observations to be made. In all but one of the patients with hypercholesteremia, the S_f 0–12 lipoproteins were elevated. The one exception (Case 34A) had lipoprotein levels at forty-eight years of age which were normal or near normal, despite a serum cholesterol concentration of 324 mg. per cent. On the other hand, an eleven year old boy (Case 32A) had definitely elevated 0–12 lipoproteins (589 mg. per cent) in the presence of a serum cholesterol level of 334 mg. per cent. A sixty-eight year old woman (Case 40A), showed a relatively slight increase in the 0–12 lipoproteins, although her serum cholesterol level was increased to 360 mg. per cent. In a number

of other cases it was similarly observed that the serum cholesterol and the S_f 0–12 lipoproteins did not parallel one another insofar as the degree of increase was concerned.

The S_f 12–20 lipoproteins were increased in most cases, although there were some rather notable exceptions (Cases 10A, 12A, 24A, 26A, 31A and 33A). These fell within the normal range as outlined for the "standard" lipoproteins by Glazier et al. In slightly more than half the cases in which lipoprotein results are available the S_f 20–100 lipoproteins were increased, but in almost as many levels were normal or near normal. The S_f 100–400 lipoproteins were normal in all but one case. The significance of the lipoprotein results as they relate to xanthomatosis and coronary artery disease will be dealt with later in this paper.

"*Borderline*" *Group* (*Table* III). As mentioned earlier, this group included all those cases in which the serum cholesterol level was between 250 and 300 mg. per 100 ml. on any single occasion, even if other determinations proved to be normal. There were twenty-five cases in this group, twenty-four from kindred I and one from kindred II. There were twelve males and twelve females. Their ages ranged from twenty-two to seventy-three years. Five (three females, two males) were spouses. The presence of vacillating cholesterol levels made evaluation of some members of this group difficult. One patient (Case 3B) showed, over a short interval of time, remarkably stable cholesterol values, ranging from 249 to 258 mg. per cent. Three years later, however, two readings made eight days apart were 298 and 283 mg. per cent, respectively. Another patient (Case 7B) had cholesterol levels of 299 and 292 mg. per cent, these determinations having been carried out some twenty months apart. After a further lapse of fourteen months, during which there was no change in dietary pattern, the serum cholesterol dropped to 256 and 263 mg. per cent (there was a thirteen day interval between these two determinations). The serum cholesterol in Case 6B dropped from 297 to 237 mg. per cent in a ten-month period, possibly due to a rather rigid low fat diet during this time. In seven patients (Cases 9B, 10B, 11B, 12B, 15B, 17B and 19B), the serum cholesterol showed fluctuations from normal to borderline levels. One of these (Case 19B) was in advanced congestive heart failure with congestive hepatomegaly and impaired liver function when first seen. The low initial serum cholesterol level may have been due to hepatocellular dysfunction, with a subsequent rise as his cardiac and hepatic functions improved. A recent serum cholesterol determination, taken at a time when liver function tests showed only minimal changes, was 202 mg. per cent.

As mentioned earlier, it is difficult and, in most cases, impossible to evaluate decisively the significance of a "borderline" serum cholesterol level. Certainly, a single determination which falls in this range should not be relied upon as the sole criterion for establishing a patient's cholesterol pattern. In many patients cholesterol levels have fluctuated between normal and "borderline" and, as shown by Wilkinson [*21*], in some patients with particularly labile cholesterol levels, they may fluctuate between normal and unequivocally hypercholesteremic values. However, when multiple determinations have been carried out in middle aged or elderly persons, and they consistently fall within "borderline" levels, or lower, one is justified, I believe, in looking upon such results as being normal. On the other hand, patient 7B whose serum cholesterol, between the ages of twenty-eight and thirty varied from 263 to 299 mg. per cent, is probably hypercholesteremic. Genetic considerations tend to lend support to this view, since both parents of this patient were hypercholesteremic; the father, in addition, had widespread tendon xanthomas.

It is, of course, of no little importance to ascertain, whenever possible, the true significance of a "borderline" cholesterol concentration, i.e., whether it represents a "high" phase in an otherwise normocholesteremic person or, conversely, a "low" phase in a hypercholesteremic subject. Thus, in genetic studies of the hypercholesteremic trait, it may be impossible, on the basis of single determinations, to conclusively designate an individual as normo- or hypercholesteremic. It is my impression that usually, but not invariably, the answer will become apparent after the serum cholesterol determinations have been repeated three or four times. Thus, the normocholesteremic person will be found to have values that swing between "borderline" and normal levels. From the experience of others, it would seem that the person who is truly hypercholesteremic may show some "borderline" levels, but most determinations will be in the hypercholesteremic range. A group is left in whom cholesterol levels remain consistently in the "borderline"

zone, and age considerations may prove to be of importance in classifying such cases (*vide supra*).

In general the "borderline" group showed the same parallelism between the serum cholesterol, lipid phosphorus and total lipids as was demonstrated in the hypercholesteremic group.

Lipoproteins in the "Borderline" Group. Lipoprotein results were normal or near normal in the majority of cases, as judged by comparison with the standard lipoprotein values reported by Glazier et al. In case 7B the patient had apparently high S_f 0–12 values; the S_f 12–20 lipoproteins were normal, as were the S_f 100–400 moieties. The S_f 20–100 levels were elevated on one occasion and normal on a second. In Case 14B the patient had high 0–12 levels, while the other lipoprotein fractions were normal. In Case 1B, the 20–100 lipoproteins were perhaps slightly elevated; this group of lipoproteins was also probably elevated in Case 2B.

Normocholesteremic Group. There were thirty-seven cases: twelve males and twenty-five females, ranging in age from five to eighty-one years. Nine were spouses. Very few of these have had multiple lipid determinations. When this has been done, the cholesterol results show a rather marked degree of constancy (Cases 1C, 6C, 7C, 11C and 13C). The lipoproteins, in those patients in whom results were available, were normal throughout, with the exceptions of slight elevation of the S_f 20–100 and 100–400 fractions in Cases 12C and 20C.

CLINICAL DATA

Xanthomatosis and Arcus (*Table* v). Tendon xanthomas occurred in twenty-three (twelve male, eleven female) of the forty-one hypercholesteremic patients. They made their first appearance generally between the ages of twenty-five and thirty-five, although in one case the onset was in the early teens. The sites of predilection for these xanthomas were, as has been reported by others [*1,5,28*], the extensor tendons of the fingers, the patellar tendons, and the tendo Achilles. The triceps tendon near its insertion into the olecranon was less frequently involved. These lesions involved the tendons and not the tendon sheaths. In a number of cases, there was involvement of sites which, to my knowledge, have not hitherto been described; the peroneal tendons just posterior to the external malleolus, the posterior tibialis tendon, the extensor hallucis longus and occasionally the extensor tendons of other toes. In addition to these tendon xanthomas, lesions (sometimes of considerable size) arising from the plantar aponeurosis (Fig. 1A) have been observed in a few cases. The fascial site of origin of the latter lesions was proved at autopsy in one case. Small lesions arising from the fascia, or, in some instances, the periosteum over the lower one-third of the tibia have been observed in six patients. (Fig. 1B.) Two patients presented rather marked prominence and irregularity of the internal malleoli, which appeared to be bony in consistency. At autopsy in one of these patients there were rather large, hard xanthomas originating in the periosteum of the internal malleoli. Thus

TABLE V

INCIDENCE OF XANTHOMATOSIS, XANTHELASMA AND ARCUS IN HYPERCHOLESTEREMIC CASES

Case No.	Patient's Age (yr.) and Sex	Total Cholesterol* (mg. %)	Tendon, Fascial and Periosteal Xanthomas	Plain and Tuberous Xanthomas	Xanthelasma	Arcus
1A	58, M	495	+	0	0	+
2A	55, M	452	+	0	0	+
3A	41, M	500	+	0	0	+
4A	43, F	517	+	0	0	0
5A	68, F	676	+	+	+	+
6A	42, M	380	+	0	0	+
7A	61, F	528	+	0	+	+
8A	35, M	632	+	0	0	+
9A	68, M	440	0	0	0	+
10A	32, F	424	+	0	0	+
11A	8, M	396	0	0	0	0
12A	19, M	380	0	0	0	0
13A	46, M	600	+	0	0	+
14A	58, M	334	0	0	0	+
15A	27, F	417	+	0	0	+
16A	40, M	568	+	0	0	+
17A	28, M	454	+	0	0	+
18A	32, M	438	0	0	0	0
19A	63, F	820	+	0	0	+
20A	30, F	494	+	0	+	0
21A	34, F	652	+	0	+	0
22A	37, F	860	+	+	+	0
23A	35, M	510	+	0	0	+
24A	64, F	544	+	0	+	+
25A	37, M	452	0	0	0	+
26A	44, M	314	+	0	0	0
27A	11, M	340	0	0	0	0
28A	9, F	325	0	0	0	0
29A	55, M	426	+	0	0	+
30A	3, F	393	0	0	0	0
31A†	55, F	343	0	0	0	+
32A	11, M	334	0	0	0	0
33A†	69, F	343	0	0	0	0
34A	48, F	324	0	0	0	0
35A	35, M	375	+	0	0	+
36A	28, F	378	0	0	0	0
37A	34, F	556	+	0	0	0
38A	9, M	329	0	0	0	0
39A	45, F	336	0	0	0	0
40A†	68, F	360	0	0	0	+
41A	6, F	440	0	0	0	0
	Total	...	23	2	6	22

* Initial serum cholesterol determination.
† Spouse.

1A 1B

FIG. 1. A, xanthomas of plantar aponeurosis (see text). B, Periosteal xanthomas arising from lower tibia. Note also involvement of patellar tendons.

it is clear that one should distinguish between fascial, periosteal and tendon xanthomas. In one case, a slight degree of calcification in xanthomatous deposits of the tendo Achilles was demonstrated roentgenographically.

Despite the often marked enlargement and nodularity of the involved tendons, there were usually no symptoms associated with these xanthomas. However, several patients mentioned that they were subject to recurrent attacks of pain in the back of the heel, sometimes associated with swelling and redness. Each of these patients had xanthomas involving the tendo Achilles. It seems likely that these attacks were due to acute bursitis resulting from abnormal pressures from the hypertrophied, nodular tendons. Pressure from footwear was probably a contributing or initiating factor.

Thannhauser [*1*] states that arthritic symptoms (joint pain and swelling) occur not infrequently in the vicinity of tendon xanthomas, and that actual osteoarthritis may develop. Such manifestations have not been observed in any of the cases in this series.

Confirmation of the xanthomatous nature of these lesions was obtained by biopsy of the tendon in three cases, and by necropsy in another, as mentioned earlier.

Two patients, both female, had cutaneous xanthomas in addition to tendon and fascial xanthomas. One (Case 5A) had only a single, small tuberous lesion over one elbow. The other (Case 22A) had plain xanthomas over the sternum and palmar aspects of the hands in addition to several small, tuberous lesions over the tendo Achilles. This patient, a member of kindred II, had very pronounced xanthelasma, forming large, disfiguring plaques on the upper and lower lids. Two sisters (Cases 20A and 21A) also had xanthelasma, although of much lesser extent. Three members of kindred I, all females, were afflicted with xanthelasma of moderate degree. In contrast to the hypercholesteremic members of kindred II in whom this lesion developed early in the fourth decade, in the affected members of kindred I xanthelasma did not develop until the sixth decade or later.

Generally speaking, xanthomas seemed to be more prone to develop in the subjects with the highest serum cholesterol levels but this rela-

tionship was far from absolute. Thus, one patient (Case 26A) whose serum cholesterol was 314 mg. per cent at forty-four years of age, had tendon xanthomas, while another (Case 9A), aged sixty-eight years, had no xanthomas despite a serum cholesterol level of 440 mg. per cent. Xanthomas are by no means an inevitable concomitant of severe or long-standing hypercholesteremia, even in families such as these in which the hereditary predisposition is particularly strong. Conversely, however, it would appear that xanthomatosis may occur in the presence of serum cholesterol levels of less than 300 mg. per cent, as has been reported by Welin and Crame [*22*]. Such an occurrence has not been observed in the present series; no instances of xanthelasma, cutaneous or tendon xanthomas have been encountered in any of the "borderline" or normocholesteremic cases. In those cases in which xanthomas have been reported to occur in the presence of random serum cholesterol levels of less than 300 mg. per cent, it is possible that repeated lipid determinations would show truly hypercholesteremic levels.

Lipoproteins and Xanthomatosis. Gofman et al. [*26*] have distinguished between xanthoma tendinosum and xanthoma tuberosum on the basis of the lipoprotein patterns. In the former, they reported that the greatest increase was in the standard S_f 0–12 lipoproteins, the next greatest increase being in the S_f 12–20 fraction and the smallest degree of increase in the S_f 20–100 group. In xanthoma tuberosum, the S_f 20–100 and 100–400 lipoproteins showed the largest increase; the S_f 12–20 lipoproteins were elevated, but to a much lesser degree; the S_f 0–12 fraction was, if anything, lower than normal.

In general, lipoprotein patterns in the present study were in accordance with Gofman's observations, insofar as they pertain to tendon xanthomas. However, I was unable to detect any significant or uniform difference between the lipoprotein results in those who had hypercholesteremia plus tendon xanthomas and those who had hypercholesteremia alone. Moreover, in contradistinction to Gofman's findings concerning xanthoma tuberosum, the two patients in our series with this type of lesion (Cases 5A and 22A) had very high S_f 0–12 and low S_f 100–400 lipoproteins, while the S_f 12–100 fraction was only moderately elevated. Admittedly both patients had tendon xanthomas as well, but since tendon and cutaneous xanthomas each appear to be but different expressions of a common metabolic disorder, there seems to be little justification for postulating a distinctive lipoprotein spectrum in each. The differences which have been reported by Gofman are based upon relatively small numbers of cases and thus may not take into account the full scope of individual variation.

Arcus Juvenilis/Senilis. It is known that this lesion consists of lipid deposits, chiefly cholesterol, which, in advanced cases, involve all layers of the cornea. In a sense, its relationship to serum cholesterol levels is somewhat analogous to that of coronary artery disease, since each is most frequently found in the absence of clear-cut hypercholesteremia (arcus being, of course, a rather common concomitant of the aging process). Yet, notwithstanding this observation, the incidence of each is enhanced in hypercholesteremic states. However, an increased incidence of arcus is not an obligatory concomitant of hypercholesteremia, Wilkinson et al. [*3*] having encountered no instances of this phenomenon in thirty hypercholesteremic members of a single family. Welin and Crame [*22*], on the other hand, found arcus in twenty-six of fifty-eight patients with hypercholesteremia. Boas [*29*], in a study of data on 1,000 consecutive cases of coronary artery disease, concluded that there was a parallel between the incidence of arcus and this type of heart disease and stated that he had noted a similar association between familial hypercholesteremia and arcus.

In the present series, arcus was detected in twenty-two of the forty-one hypercholesteremic subjects. There were eleven under the age of fifty, eight of these being forty or less. The youngest was twenty-seven years of age. As noted earlier, three of the hypercholesteremic patients were spouses of members of kindred I. None of these had xanthomatosis, but arcus was present in two.

Only two "borderline" and two normocholesteremic subjects had arcus. With one exception, (Case 12C), a forty-seven year old male who had barely perceptible arcus, these subjects were in the seventh decade of life.

CORONARY ARTERY DISEASE

Hypercholesteremic Cases (*Table* VI). Fourteen of the forty-one hypercholesteremic patients (nine males, five females) had clinical coronary artery disease. Of this number, one (Case 14A) had had a myocardial infarction at fifty-five

TABLE VI
ELECTROCARDIOGRAPHIC RESULTS IN HYPERCHOLESTEREMIC SUBJECTS WITH CLINICAL CORONARY DISEASE

Case No.	Patient's Age (yr.) and Sex	Clinical Coronary Disease	Age (yr.) at Onset	Electrocardiogram (Patient Resting)	2-Step Test
1A	58, M	+++	57	N	+
2A	58, M	+++	35–40	N	+
3A	41, M	+	40	Ab	..
5A	69, F	+	63	N	+
6A	45, M	+++	41	?Ab	+
7A	63, F	+	57	?Ab	+
9A	69, M	+++	59	Ab	..
13A	48, M	+	41	N	+
14A	58, M	++	54	Ab (infarct)	..
19A	63, F	+	60	Ab	..
24A	64, F	+	62	N	+
29A	55, M	++	52	Ab (infarct)	..
35A	35, M	+	35	Ab	..
40A*	68, F	+	48–50	Ab	..

NOTE: + = Angina.
++ = Myocardial infarct.
+++ = Fatal coronary occlusion.
?Ab = Borderline abnormal.
N = Normal.
* Spouse.

years of age, from which he recovered without angina subsequently developing. The remaining thirteen patients had angina. In one instance (Case 29A) this symptom began following a myocardial infarction at fifty-two years of age. Four of the fourteen patients mentioned herein died of coronary occlusion during the course of this investigation, after having suffered from angina for varying periods of time. It will be noted that one of the three hypercholesteremic spouses was also a victim of this syndrome. The six patients who had coronary occlusions (two of whom survived) were all males.

The earliest age of onset of clinical coronary disease, as expressed by angina pectoris, in this series was thirty-five years. In five cases (four males, one female) it began in the fifth decade, in four (three males, one female) in the sixth decade, and in four (three females, one male) in the seventh decade. Although the number of cases is admittedly small, it is clear that, in this series, coronary disease tended to develop at an earlier age in the males than in the females.

These figures, dealing as they do only with subjects who were available for examination and study during the three years of this project, do not provide a true reflection of the incidence of coronary artery disease in these families. Not included in the data are many siblings and forebears whose mode of death strongly suggests coronary occlusion.

Electrocardiograms were obtained in thirty-two cases, including the fourteen patients with clinical coronary disease. Two patients (Cases 31A and 33A) were spouses. In twenty-three patients electrocardiograms were obtained after exercise employing the Master two-step test. The single two-step test was used in three cases, the double two-step test in twenty. In many instances the test was carried out in the patients' homes, making use of a lightweight two-step device which was built to Master's specifications [*30*]. In all cases, the three standard limb leads, plus one (more often two or three) precordial leads—either V4, or V4 and V5, or V2, V4 and V6, were used in the electrocardiogram which was taken after exercise. Recently, it was decided to employ leads V3, V4 and V5 routinely. Before exercise, the customary twelve-lead electrocardiogram was carried out. Until recently, Master and his associates used the standard limb leads plus V4 or V5 after exercise. At present, this group advocates using only lead II of the limb leads, in combination with three precordial leads (V3, V4 and V5 or V4, V5 and V6), since they believe that the precordial leads are more informative [*31*]. We have not considered it expedient to change our methods at this time, and it is proposed to continue using the standard limb leads for the time being, in addition to V3, V4 and V5.

Results. In the fourteen cases of clinical coronary disease the results were as follows: four patients had normal electrocardiograms before exercise; one (Case 24A) was classified as probably normal (there was a slight ST segment sag in leads II and V6 in this case). In seven cases, the electrocardiogram before exercise was abnormal, showing evidence of old infarction in two, in the others demonstrating the non-specific changes usually attributed to chronic coronary insufficiency. In the remaining two cases, there was slight sagging of the ST segment below the baseline in one or more leads (usually leads I, II and the left precordial leads). These cases were designated as "borderline abnormal . . . " Burch [*32*] has recently emphasized that flattening and/or lowering of the ST segment may be the earliest electrocardiographic changes of coronary disease, if one has excluded such factors as smoking, eating, standing and exposure to cold.

The four patients in whom electrocardiograms taken when resting were normal and the one with a "probably normal" electrocardiogram had abnormal two-step tests (three had

double two-step tests; two had single two-step tests). In these patients there was a RS-T segment depression of from 0.75 mm. to 4 mm. Master and his associates [*31*] state that a RS-T drop after exercise of greater than 0.5 mm. in any lead is abnormal and indicative of coronary insufficiency. In support of this contention they cite their own extensive experience and that of Mattingly et al. The latter group followed 1,000 persons suffering from chest pain over a period of an average of seven years, using the Master two-step test as a diagnostic and prognostic aid. In their view, a RS-T depression of 0.5 to 1.0 mm. was found to be as effective an indicator of coronary artery disease as was a depression of 1 to 2 mm. This was based upon the finding of an equal incidence of fatal and non-fatal coronary thrombosis in the two groups—those who had 0.5 to 1.0 mm. depression and those who had depressions of 1 to 2 mm. Notwithstanding these findings, there are many dissenting opinions regarding these criteria, some believing that the RS-T depression should be at least 1 mm., and others insisting on a downward shift of 2 mm. or more.

In a recent review of this subject, Feuiltault [*33*] acknowledged the usefulness of the test, but held that a 1 mm. RS-T depression must be present for the electrocardiogram to be considered abnormal. However, he added that a RS-T depression of 0.5 mm., combined with a "flat" ST shape or a "sagging" ST without any ST depression following exercise should also be considered abnormal. In reviewing our two-step electrocardiograms, we too have concluded that such changes in the ST configuration after exercise are of diagnostic importance. However, for the sake of uniformity we are adhering to Master's basic criteria in defining abnormalcy, and hence RS-T depressions of greater than 0.5 mm. have been designated as abnormal.

Two-step tests (1 double, 1 single) were carried out in the two patients whose electrocardiograms taken when resting were considered to be "borderline abnormal." In each case the electrocardiogram taken after exercise was abnormal, showing 1 mm. RS-T depressions.

Thus, all fourteen patients with overt coronary disease showed electrocardiographic abnormalities, either in the electrocardiogram taken when resting or after the Master two-step test.

Electrocardiograms were obtained in eighteen patients with no angina. (Table VII.) Two-step tests (fifteen double, one single) were carried out in all but two of these. The electrocardiogram taken with the patient at rest was normal in sixteen cases, abnormal in one (Case 33A, a patient with severe hypertension of long standing whose electrocardiogram showed changes compatible with myocardial ischemia) and "borderline abnormal" (i.e., slight sag of the ST segment in one or more leads) in the other. The two-step test was positive in nine cases, including the patient whose electrocardiogram taken when resting was "borderline abnormal." In one case, the two-step test result was questionable, the RS-T depression being just 0.5 mm., and not accompanied by sagging or flattening of the ST segment. In one patient (Case 8A) a double two-step test carried out at thirty-five years of age was normal, while a second test, at age thirty-seven, was abnormal (ST depression of 1 mm. in V4). However, the reverse of this was seen in another patient (Case 22A) whose two-step test was abnormal (ST depression between 0.5 and 0.75 mm.) at age thirty-seven, but normal when repeated a year later. In Case 4A there has been a definite deterioration in the pattern after exercise during the three years the patient has been under observation. In the electrocardio-

TABLE VII

ELECTROCARDIOGRAPHIC RESULTS IN HYPERCHOLESTEREMIC SUBJECTS WITHOUT CLINICAL CORONARY ARTERY DISEASE

Case No.	Patient's Age (yr.) and Sex	Electrocardiogram (Patient Resting)	2-Step Test
4A	43, F	? Ab	+
8A	37, M	N	+
10A	32, F	N	N
12A	19, M	N	+
15A	27, F	N	+
16A	40, M	N	+
17A	28, M	N	N
18A	33, M	N	N
20A	30, F	N	N
21A	34, F	N	N
22A	37, F	N	+
23A	35, M	N	+
25A	37, M	N	+
26A	44, M	N	N
31A*	59, F	N	+
33A*	69, F	N	N
34A	48, F	N	N
39A	45, F	N	..

* Spouse.

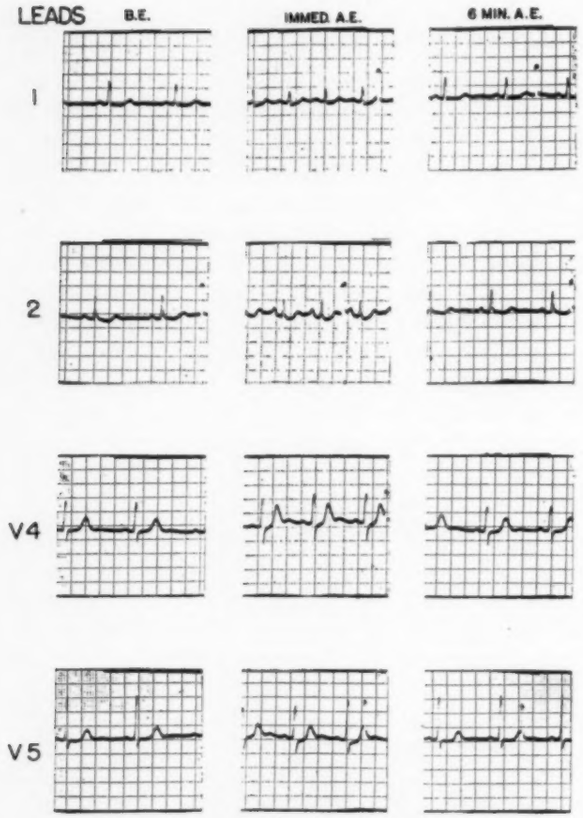

FIG. 2. Before exercise, this electrocardiogram, that of a forty-three year old, asymptomatic woman with hypercholesteremic xanthomatosis, showed sagging of the RS-T segment in leads 1, 2, AVL, V5 and V6 (these changes have been designated as "borderline abnormal"). After the double two-step test, significant RS-T depressions appeared. At six minutes the electrocardiogram had reverted to its pre-exercise appearance. The two-minute electrocardiogram is not shown. B.E. = Before exercise. A.E. = After exercise.

gram taken with the patient at rest there was a slight sag of the ST segment in leads I, II, AVF, V5 and V6. (Fig. 2.) After the double two-step test (which elicited only slight dyspnoea) definite RS-T depressions appeared and there was an intensification of the ST sag.

It is impossible to state at this juncture whether or not this test may be considered to have diagnostic or predictive value in hypercholesteremic subjects who do not present symptomatic or objective evidence of coronary disease. Some elucidation of this problem may become apparent in the course of long-term follow-up studies of these patients, making use of the normocholesteremic and, perhaps, some of the "borderline" relatives as control subjects.

Wener et al. [*34*], in a study of 311 apparently normal subjects reported false positive single or double two-step tests in 8.0 per cent. Master, Field and Donoso [*31*], in a comparable type study of 250 patients with definite coronary artery disease, found that, although 142 patients had false negative single two-step tests, only eight patients (3.2 per cent) had false negative single and double two-step tests. If one were to apply these observations to the results obtained in the present study, it would be tempting to conclude that 90 per cent of those who had positive two-step tests despite the absence of angina (i.e., nine of the hypercholesteremic patients) were suffering from occult coronary artery disease. However, Master's findings have been challenged by a number of workers who have reported somewhat higher percentages of false-positive results [*35*]. Nevertheless, the percentage of positive results reported herein is sufficiently high that one is justified in maintaining a high index of suspicion that chronic, asymptomatic coronary insufficiency exists in some of these cases. As mentioned earlier, continued observations on these and additional cases may well shed further light on the value of the two-step test in presymptomatic coronary disease.

"*Borderline*" *Cases.* Angina was not present in any of the twenty-five patients with "borderline" cholesterol levels. Electrocardiograms were obtained in twenty cases and in one the electrocardiogram was obtained elsewhere. This was reported to be normal by the patient's physician. Including this patient, there were five who had electrocardiograms taken at rest only. In three patients the electrocardiograms were normal; in one patient (Case 19B) the electrocardiogram revealed changes consistent with myocardial ischemia and an old posterior infarct (the latter was confirmed at autopsy). Another patient (Case 5B), with hypertension, showed electrocardiographic changes consistent with the so-called "left ventricular strain" pattern.

In sixteen cases, the two-step test was carried out (two single, fourteen double). In nine (two single, seven double) there were no significant electrocardiographic changes after exercise. In one case (Case 16B) the RS-T depression was no greater than 0.5 mm.; this was looked upon as a questionably abnormal response. In the remaining five cases the electrocardiograms showed RS-T depressions of from 0.75 to 1.0 mm.; these were considered to be abnormal responses to exercise. The ages of the patients who showed

this type of response were twenty-eight, thirty-seven, thirty-eight, forty-two and sixty-six years. This is a small number of cases, and one should not attempt to read too much into the results. Notwithstanding this, the percentage of positive results does appear to be excessively large. This may be interpreted as indicating that the incidence of false positive results is high or that some of these patients are suffering from occult coronary artery disease. One is led again to speculate on the significance of the finding of borderline cholesterol levels in young adults. As mentioned earlier, it would seem that, in some cases at least, cholesterol values of this order might actually indicate hypercholesteremia. If such is the case, it might be anticipated that in some of these subjects coronary artery disease would be prone to develop in the same way as in those with indisputable hypercholesteremia.

Normocholesteremic Cases. These numbered thirty-seven, of which nine were spouses. Electrocardiograms were obtained in thirteen cases including three spouses; in seven of these double two-step electrocardiograms were carried out. Only one patient (Case 37C) gave a clear-cut history of coronary artery disease. This patient had an episode of chest pain characteristic of a coronary occlusion at about sixty-five years of age, and the electrocardiogram showed myocardial ischemia and evidence of an old posterior myocardial infarct. In Case 2C there was a history of a rather prolonged episode of anterior chest pain at sixty-seven years of age. Since she was afflicted with severe widespread rheumatoid arthritis, it was not possible to exclude this as the source of her pain. Her electrocardiogram was normal. The exercise test was not carried out. Another patient (Case 36C), aged thirty-five, the son of the two aforementioned subjects, had symptoms which were suggestive but not diagnostic of angina; a double two-step test was positive. None of the other normocholesteremic subjects had angina. Of the ten electrocardiograms obtained in the latter group, one patient (Case 1C) showed abnormalities due to a severe myocarditis of unknown etiology from which he had made an almost complete recovery. In four patients electrocardiograms taken at rest were normal; in four patients electrocardiograms, taken with the patients at rest and after the double two-step test, were normal; and in two patients electrocardiograms, taken at rest, were normal but those taken after the double two-step test were abnormal (RS-T depression of 0.5 to 0.75 mm. in one [Case 7C], and 1.0 mm. in the other [Case 10C]). The latter two patients were aged thirty-five and thirty-one years, respectively.

In view of the fact that the lipoprotein data are incomplete, it has not been possible to arrive at any worthwhile conclusions regarding the relationships between the serum cholesterol and lipoprotein concentrations insofar as they pertain to the presence or absence of clinical coronary artery disease. Certainly, there does not seem to be any indication in the results obtained thus far of a superiority of one modality over the others in indicating the presence of coronary disease. Rather, it would seem that they parallel one another in a rough sort of way and that, while the presence of hypercholesteremia and hyperlipoproteinemia may indicate a greater mathematical probability of the occurrence of coronary artery disease in any given person, the presence of normal cholesterol and lipoprotein concentrations obviously does not rule it out. In Case 19B for example, both the serum cholesterol and lipoprotein levels were normal or "borderline," yet the patient had had an old posterior infarct and was found, at autopsy, to have advanced atheromatous disease of the coronary arteries.

Long-continued observations of the normal and hypercholesteremic members of these two families, with repeated clinical evaluation, and simultaneous determinations of the serum lipids and lipoproteins may ultimately make it possible to ascertain more clearly the diagnostic and prognostic potentialities of the serum cholesterol versus the lipoprotein indices with respect to coronary artery disease. I say this in the full awareness that in general population studies neither determination appears to be very useful in predicting which persons are eventually to fall prey to coronary artery disease. In groups of hypercholesteremic subjects, however, as has been demonstrated in this and in many other papers, there is a sharp increase in the incidence of coronary disease and it is in such groups that comparative studies of this sort might prove rewarding. In this regard, the reader is referred to the report of the cooperative study initiated by the National Advisory Heart Council in the U.S.A. [*11*], in which large numbers of presumably normal subjects were studied from the standpoint of determining whether, among those in whom myocardial infarction developed within

a two-year period from the time of inception of the study, the S_f 12–20 and 20–100 lipoproteins or the cholesterol concentrations gave a better degree of predictability. The majority of the participating laboratories concluded that, although both were elevated in some of the patients in whom myocardial infarction subsequently developed, neither was superior to the other in its predictive value. It should be noted that the Donner Laboratory group dissented from this view, holding that the S_f 12–20 and 20–100 lipoproteins did indeed show a better correlation with the subsequent appearance of myocardial infarction than did the cholesterol concentrations.

It is sometimes said that familial hypercholesteremia, in addition to predisposing to the development of coronary artery disease, has similar effects with respect to peripheral vascular and cerebrovascular disease. This is not borne out by most reports in the literature nor was any substantiation for this opinion elicited by the study under discussion.

One patient, Case 33A (a spouse), suffered from cerebrovascular disease, manifested as recurrent strokes, with onset at sixty-nine years of age. Severe essential hypertension was considered to have been the etiologic factor in this case. No other instances of cerebrovascular disease were elicited. Another spouse (Case 40A) suffered from arteriosclerosis obliterans, as manifested by intermittent claudication. This patient, with hypertension of somewhat lesser degree, was sixty-eight years of age. None of the remaining hypercholesteremic patients presented clinical evidence of either cerebrovascular or peripheral vascular disease. Roentgenographic studies of the pelvis and legs for evidence of arterial calcification were available in only fourteen hypercholesteremic cases. One showed calcification of the abdominal aorta; one had slight calcification of the femoral arteries; one (Case 24A, a diabetic patient) showed faint calcification of the femoral and tibial arteries; one (Case 40A, *vide supra*) showed rather marked calcification of the iliac, femoral and popliteal arteries. All four patients were in their seventh decade and, interestingly enough, all were females (a reversal of the sex ratio which was observed in coronary artery disease). In the other ten cases, there was no evidence of arterial calcification.

None of the patients considered "borderline" cases had symptomatic or clinical evidence of peripheral vascular or cerebrovascular disease. X-ray studies for arterial calcification were carried out in only seven cases. In three (one male, aged sixty-eight; two females, aged seventy and fifty-one years), slight degrees of calcification were detected in either the pelvic or tibial arteries, or both. In four cases, no arterial calcification was observed.

One normocholesteremic patient (Case 37C, a spouse), a seventy-two year old male, was found to have a large calcified abdominal aortic aneurysm; pedal pulses were absent. None of the other patients in this group evidenced either peripheral vascular or cerebrovascular disease. One, a sixty-seven year old female, showed minimal calcification of the femoral arteries. In the three others in whom x-ray studies were made, no vessel calcifications were seen.

These numbers are admittedly small, but on clinical grounds alone, I think one is justified in concluding that neither peripheral vascular disease (arteriosclerosis obliterans) nor cerebrovascular disease seems to bear any intimate relationship to hypercholesteremia. A more direct relationship is seen with respect to hypertension and diabetes.

The incidence of essential hypertension was low. Of thirty-seven hypercholesteremic patients whose blood pressures were obtained, only five had diastolic pressures of 100 or more. Blood pressures were obtained in twenty "borderline" cases; in three, the diastolic pressure was 100 or over. All twenty-three normocholesteremic subjects whose blood pressures were obtained had diastolic levels well below 100.

As mentioned earlier, one hypercholesteremic patient had coexisting mild diabetes. Blood sugar determinations were carried out in thirteen additional cases and were normal in each instance. None of the other patients, hypercholesteremic or otherwise, presented clinical evidence of diabetes mellitus. In addition to Case 19A, in which a diagnosis of myxedema had earlier been firmly established, one other patient (Case 2C) was found to have a low basal metabolic rate in the presence of symptoms and signs compatible with mild hypothyroidism. This patient was normocholesteremic. There was no clinical evidence of hypothyroidism in any of the other cases; because of this, relatively few determinations of the basal metabolic rate have been carried out.

In view of the occasional appearance in the medical literature of reports dealing with

xanthomatous involvement of heart valves, chiefly the aortic and, less frequently, the mitral and pulmonic valves, the incidence and distribution of heart murmurs in this series was examined. Cardiac auscultation was carried out in thirty-nine of the hypercholesteremic subjects. Heart murmurs were present in eighteen patients, including two spouses. Seven had aortic systolic murmurs whose intensity was graded 1 or 2. An additional patient had both aortic and mitral systolic murmurs which were thought to be due to rheumatic valvular heart disease. In the ten remaining patients, the systolic murmurs were chiefly pulmonic in locale; a few were maximal at the apex. In these ten, therefore, one could not rule out so-called physiologic heart murmurs. Since not all aortic systolic murmurs, especially when of such low intensity, indicate aortic stenosis, and since aortic stenosis itself is most often due to other causes; these results, of course, are not evidence *per se* of a high incidence of xanthomatous aortic stenosis. Like other facets of this disorder, observations made over an extended period of time may make clear what importance should be attached to the finding of such murmurs.

In the "borderline" group, cardiac auscultation was carried out in twenty cases. Three patients were found to have grade 1 or 2 aortic systolic murmurs. Of twenty-four normocholesteremic patients in whom auscultation was carried out, two proved to have grade 1 aortic systolic murmurs. The finding of these murmurs in normocholesteremic and "borderline" subjects reaffirms the remarks made herein regarding xanthomatous aortic stenosis.

It is perhaps worthy of note that, in the only hypercholesteremic patient to come to necropsy, quite advanced atheromatous deposits were found in the pulmonary arteries (Fig. 3), despite the absence of chronic pulmonary disease, or of any pathologic condition which might have given rise to pulmonary hypertension.

With one exception, a patient (Case 22A) who was found to have coexistent pericholangiolitic cirrhosis and familial hypercholesteremia, there was no clinical or laboratory evidence of liver disease in this series. Many of the hypercholesteremic patients had rather high serum total proteins due to an elevation of the albumin component. As mentioned earlier, electrophoretic fractionation of the serum proteins and lipoproteins is under way and the findings will be reported separately.

FIG. 3. Multiple, large atheromatous deposits in the right pulmonary artery of a hypercholesteremic patient who died of myocardial infarction (see text).

Genetic and therapeutic considerations and certain observations relating to the effects of acute stress will be dealt with in later communications.

SUMMARY AND CONCLUSIONS

1. Of 103 members of two families, forty-one were found to be hypercholesteremic (thirty-eight in kindred I, three in kindred II). These included three who were spouses. Twenty-five subjects had serum cholesterol values in what has been designated a "borderline" zone (i.e., from 250 to 300 mg. per cent). Thirty-seven had normal cholesterol concentrations.

2. It is suggested that some of those whose serum cholesterol values were in the "borderline" zone might actually be hypercholesteremic.

3. Some subjects were stable and others labile with respect to the serum cholesterol values. Thus, single estimations are not always to be relied upon as a basis for determining normalcy or abnormalcy.

4. In all hypercholesteremic cases, the S_f 0–12 lipoproteins were elevated; the S_f 12–20 lipoproteins were elevated in most cases; in slightly more than half the cases in which lipoprotein results were available there was an elevation of the S_f 20–100 fraction while the S_f 100–400 lipoproteins were normal in most instances.

5. The lipoprotein values were normal or near normal in the "borderline" and normocholesteremic subjects.

6. Tendon, fascial or periosteal xanthomas occurred in twenty-three of the forty-one hyper-

cholesteremic patients. They did not occur in any of the spouses. In addition to the commonly described sites of origin, it was observed that some xanthomas originate in sites not previously described: the plantar aponeurosis, periosteum overlying the lower tibia and medial malleoli and the peroneal and posterior tibial tendons. Cutaneous xanthomas (excluding xanthelasma) occurred in two patients. Xanthelasma was observed in six patients.

7. Although the incidence of xanthomas increased with increasing age, the relationship was not absolute, some hypercholesteremic patients having reached the sixth and seventh decades without xanthomatosis developing.

8. Some correlation between xanthomatosis and the degree of hypercholesteremia was observed, but here again there were exceptions. No xanthomas were found in the normocholesteremic or "borderline" subjects.

9. Lipoprotein patterns could not be relied upon to distinguish between tendon and cutaneous xanthomas.

10. Arcus senilis occurred in twenty-two hypercholesteremic subjects, eleven of whom were less than fifty years of age. Only two normocholesteremic and two "borderline" subjects were found to have arcus.

11. Fourteen hypercholesteremic subjects had clinical coronary artery disease. Four of these died of coronary occlusion during the course of this study. All had electrocardiographic abnormalities, either in the electrocardiograms taken at rest or after the two-step test.

12. In sixteen asymptomatic hypercholesteremic patients, the Master two-step test was carried out; the response to exercise was abnormal in nine. It is suggested that, in some of these patients, this test might indicate "occult" or presymptomatic coronary artery disease.

13. Angina was not present in the "borderline" group but there was electrocardiographic evidence of an old posterior infarct in one patient. Of sixteen two-step tests, five were positive. In the normocholesteremic group, one patient had definite clinical coronary artery disease (old posterior infarct) and two were suspected of having angina. One of the latter had a positive two-step test. Two of ten asymptomatic, normocholesteremic patients who underwent this procedure had positive two-step tests.

14. The incidence of hypertension, cerebrovascular and peripheral vascular disease in this series was low. It is suggested that hypertension and diabetes are more likely to be associated with these forms of atheromatous disease than is hypercholesteremia *per se*. Age, too, is an important factor; all but one of the patients who had clinical evidence of aortic or peripheral arterial disease, or who showed calcification of pelvic or lower limb arteries on x-ray examination, were in the sixth or seventh decades.

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Studies in Essential Hypercholesterolemia and Xanthomatosis*

Relationships Between Age, Sex, Cholesterol Concentrations in Plasma Fractions, and Size of Tendinous Deposits

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A GREAT number of studies, among others those by Barr, Russ and Eder [*1*], Nikkilä [*2*], Svahn [*3*], Rosenberg [*4*], Oliver and Boyd [*5*], and Jencks and co-workers [*6*], have confirmed the existence of an increased β/α-lipoprotein ratio in patients with early clinical manifestations of atheromatosis, taken as a group. There is general agreement that there is an absolute increase in the β-lipoprotein level. Whether an absolute decrease in the level of α-lipoprotein is consistently present seems more open to doubt.

Barr, Russ and Eder [*1*] in their careful studies emphasized the finding of a relative and absolute reduction in α-lipoprotein in atherosclerosis and kindred conditions. This was particularly apparent in nephrosis and xanthoma tendinosum, although in the latter disorder their conclusions were based on only two cases, both patients were males. The later study by the same authors, in 1955, included seven cases of essential hypercholesterolemic xanthomatosis (in only one was the patient a woman) and seemed to confirm the presence of consistently low α-lipoprotein levels. Lever and co-workers [*8*], on the other hand, found a decidedly low α-cholesterol in their eight women with essential hypercholesterolemic xanthomatosis. Their figures for the four women in the reproductive period were lower than those in the group above fifty years of age.

We have over a period of many years collected reports on a large number of patients with essential hypercholesterolemic xanthomatosis; with family members, comprising several hundred individuals. Analysis of the findings in these subjects revealed a continuous spectrum, varying from subjects with extremely elevated serum cholesterol values and marked, usually symmetric tendinous deposits at a comparatively young age to subjects with a moderate increase in the serum cholesterol level and one or two small xanthomas the size of a pea or less, usually discovered at a fairly late age.

CASES STUDIED

Those patients who did not present a clear serum in the fasting state were excluded. We have used rigid criteria, excluding all cases in which there was any doubt as to the existence of characteristic nodules.

The cases studied include forty-five patients with xanthomatosis, twenty-one with essential hypercholesterolemia without demonstrable xanthomas, and healthy normal subjects; twenty-nine males, aged twenty-two to forty-five years, and twenty-three females, aged eighteen to twenty-eight years. We have excluded patients who were under active treatment; however, we accepted those on a fat-poor diet.

The present study represents only a small fraction of our over-all investigation. The younger and symptom-free members of the families included are definitely under-represented.

A few cases of nephrosis as well as of congenital adrenal hyperplasia have been brought into the study to illustrate special points.

Essential Hypercholesterolemic Xanthomatosis. The group with essential hypercholesterolemic xanthomatosis has been subdivided into three groups,

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TABLE I

RELIABILITY OF THE COMBINED METHOD OF PLASMA FRACTIONATION AND CHOLESTEROL DETERMINATION AT VARIOUS LEVELS OF CHOLESTEROL

Cohn Fraction	Cholesterol (mg. %)	Duplicate Determinations (no.)	$2 \times \alpha$ (mg. %)	$\frac{\alpha}{\sqrt{2}}$ (mg. %)
IV + V + VI	<50	84	4.48	1.58
IV + V + VI	>50	175	7.3	2.58
I + II + III	100–200	67	8.92	3.15
I + II + III	200–300	118	9.44	3.34
I + II + III	300–400	52	12.22	4.32
I + II + III	>400	27	15.52	5.49

TABLE II

CHOLESTEROL DISTRIBUTION IN NORMAL SUBJECTS
Comparison Between Eder's 1955 Series and Present Series

Series	Fraction IV + V + VI		Fraction I + III* Fraction I + II + III†	
	(mg. %)	(% of total)	(mg. %)	(% of total)
Females				
Eder's (1955), aged 19 to 35 years	60	33	123	67
Present series, aged 18 to 28 years	64	30	157	70
Present series corrected‡	61	31	139	69
Males				
Eder's (1955), aged 19 to 35 years	52	25	153	75
Present series, aged 22 to 45 years	56	26	168	74
Present series corrected*	53	26	155	74

* Eder's series.
† Present series.
‡ The values have been corrected by the average difference between the modified Theorell method used and the Sperry-Webb procedure, i.e,. 4.6 per cent. From our values for fraction I + II + III we have subtracted the average value of cholesterol in fraction II for young males and females, respectively, as found by Barr, Russ and Eder [*1*].

according to the size of their xanthomas. Grade 3 denotes multiple extensive symmetric deposits in several tendons of both hands and in both Achilles tendons and usually also in the patellar tendons, the peroneal tendons and in some of the dorsal tendons of the feet. Grade 2 denotes moderate deposits, usually symmetric in the Achilles tendons, and often asymmetric and fairly small nodules in the tendons of the hand. Grade 1 denotes the presence of isolated nodules the size of a pea or less in the dorsal tendons of the hands or of one hand.

Essential Hypercholesterolemia. We have accepted patients as having established essential hypercholesterolemia in this series only if they have been followed up for a long time and showed consistently elevated values, usually between 350 and 450 mg. per cent. Patients with essential hypertension, essential hyperlipemia, diabetes and hypothyroidism have been excluded.

The plasmas of two nephrotic subjects were fractionated without previous high speed centrifugation. They have been used in this series merely for comparison with the low values for cholesterol in fraction IV + V + VI obtained by Barr, Russ and Eder [*1*].

Congenital Adrenal Hyperplasia. One of these two patients presented marked virilizing features and high 17-ketosteroid values (subject I. A.). The other had well developed musculature, very marked hirsutism and presented moderately elevated 17-ketosteroid values (subject B. P.).

Methods. Blood was drawn in the morning in the fasting state.

Cholesterol fractionation: For each 8.5 ml. of blood 1.5 cc. of acid citrate solution (A.C.) was used. Reagents were prepared according to Lever et al. [*9*].

Fractionation procedure: The fractionation procedure was carried out according to Barr et al. [*1*]. Cholesterol was determined by a modification of the method given by Theorell [*10,11*]. This procedure has been submitted to extensive comparison with the method of Sperry and Webb [*12*], the result of which will be published separately by other members of our group (K. Cramér and B. Isaksson). At all levels of serum cholesterol the modified Theorell procedure showed a slightly higher value than the Sperry-Webb procedure, averaging +4.6 per cent.

The combined error in the fractionation and cholesterol determination procedures has been estimated by calculation of the mean error between duplicate determinations separately at various levels of cholesterol in both fractions. The mean error of the combined method of fractionation and cholesterol determination at different cholesterol levels is shown in Table I.

Barr, Russ and Eder [*1*] found a very low and reasonably constant level of cholesterol in fraction II (γ-globulin) in young women and men, averaging about 3 per cent. This level was not changed systematically in the diseases investigated. On the strength of this we have not separated fraction II from fraction I + II + III. In Table II we give our values in normal young men and women as compared with those published by Eder et al. in 1954 [*13*]. Uncorrected values are cited as well as those corrected by subtracting the

TABLE III

CHOLESTEROL IN PLASMA FRACTIONS IN DIFFERENT CLINICAL GROUPS

Group	Subjects (no.)	Average Age (yr.)	Total Plasma Cholesterol (mg. %)	Sum of Fractions (mg. %)	I + II + III (mg. %)	IV + V + VI (mg. %)	I + II + III (%)	IV + V + VI (%)
Xanthomatosis, grade 3:								
Males	3	42	496	501	464	38	92	8
Females	7	50	531	544	485	59	89	11
Both sexes	10	47	521	532	478	52	90	10
Xanthomatosis, grade 2:								
Males	7	49	398	418	365	52	88	12
Females	9	54	369	381	328	53	86	14
Both sexes	16	52	382	397	345	62	87	13
Xanthomatosis, grade 1:								
Males	16	55	323	330	283	47	86	14
Females	3	48	349	374	329	45	88	12
Both sexes	19	53	327	337	290	46	86	14
Essential hypercholesterolemia:								
Males	10	42	322	341	291	50	85	15
Females	11	42	369	379	303	77	79	21
Both sexes	21	42	347	361	296	64	82	18
Survivors after myocardial infarction:								
Males	23	54	282	297	237	59	80	20
Nephrosis:								
Males	1	73	319	316	294	22	93	7
Females	1	45	495	517	496	20	96	4
Both sexes	2	59	407	416	395	21	95	5

average values of cholesterol in fraction II as determined by Barr, Russ and Eder [*1*]. In the last column our values have also been corrected for the systematic error in our modified Theorell procedure as compared with the procedure of Sperry and Webb.

RESULTS

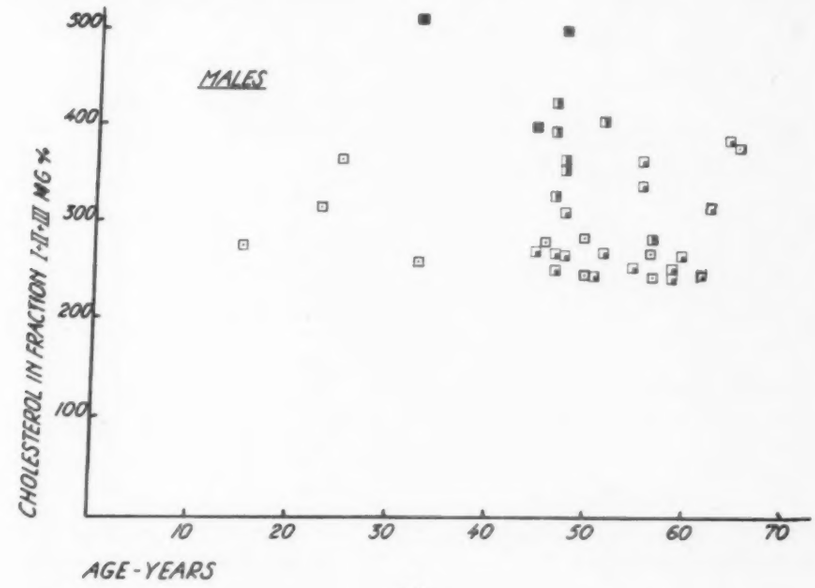
The results are summarized in Tables III and IV and Figures 1 to 4.

Figures 1A and B show the concentration of cholesterol in fraction I + II + III ($\beta + \gamma$-lipoprotein) in relation to age. Above the age of fifty there are no men with the most severe degree of xanthomatosis (grade 3), but there are a few women in this severe state at ages between fifty-five and sixty. Some women with moderate xanthomatosis (grade 2) still survive at ages between sixty-five and seventy-five. Small isolated tendinous xanthomas (grade 1) were found in quite a few subjects at forty-five to fifty years of age and with a β-cholesterol level

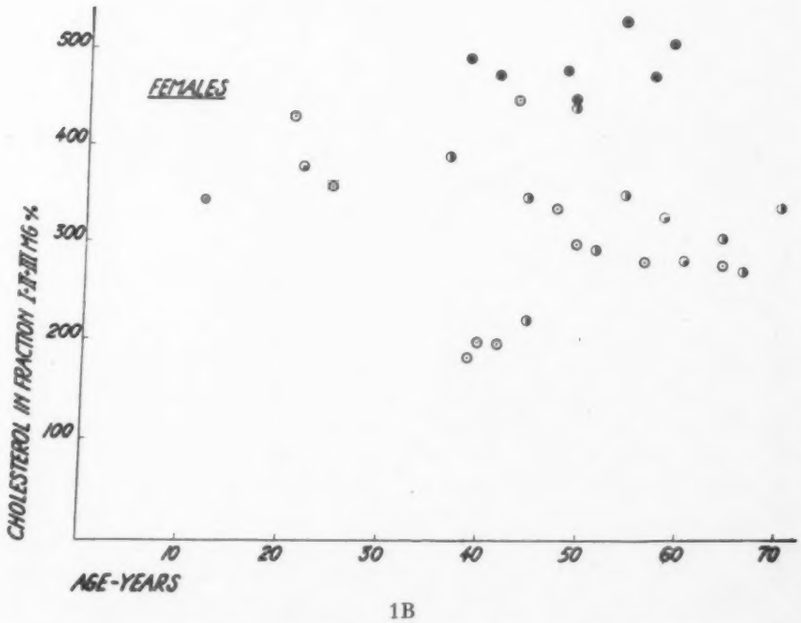
TABLE IV

COMPARISON BETWEEN CHOLESTEROL VALUES FOUND IN FRACTION IV + V + VI AND IN FRACTION IV + V

Subjects	Cholesterol (mg. %)	
	Fraction IV + V + VI	Fraction IV + V
S. O.	22	22
A. M. K.	105	101
A. L. K.	104	99
C. D.	62	54
E. O.	91	85
G. O.	53	46
S. W.	52	43
E. S.	56	43
I. T.	100	91
A. E.	37	33
S. F.	38	39



1A

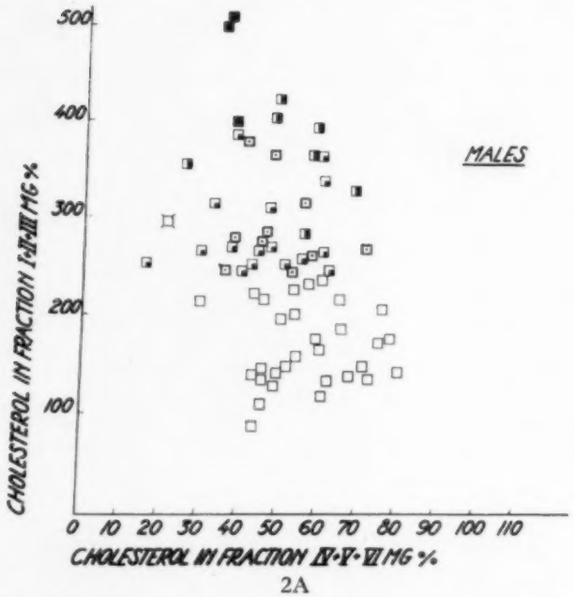


1B

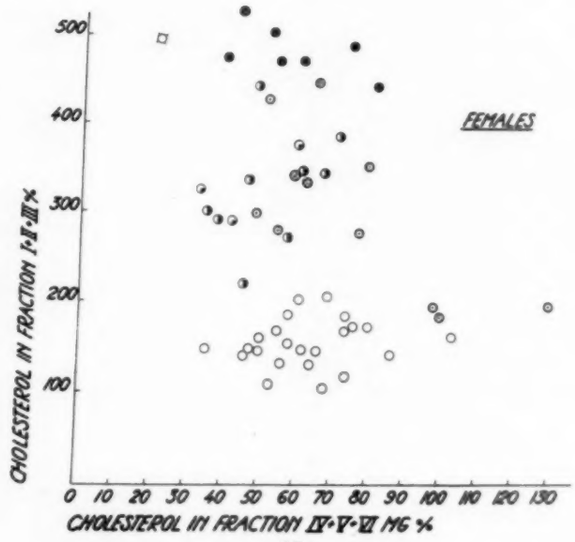
FIGS. 1A and B. Cholesterol concentration in fraction I + II + III in relation to age. Squares, males. Circles, females. ◘ ⊙ Essential hypercholesterolemia. ◪ ◔ Xanthomatosis, grade 1. ◨ ◑ Xanthomatosis, grade 2. ■ ● Xanthomatosis, grade 3.

of about 250 mg. per cent. However, in only one case in the group between fifteen to thirty years (aged twenty-one) did we find a small isolated tendinous xanthoma, although the general level of β-cholesterol in this group was quite high.

Figure 2A shows that there is a low average concentration of cholesterol in fraction IV + V + VI in essential hypercholesterolemia in the male. However, this low concentration is not obligatory and severe degrees of xanthomatosis



2A



2B

FIGS. 2A and B. Relation of cholesterol concentration in fraction I + II + III to cholesterol in fraction IV + V + VI. Squares, males. Circles, females. □ ○ Normal subjects. ◘ ⊙ Essential hypercholesterolemia. ◪ ◔ Xanthomatosis, grade 1. ◧ ◑ Xanthomatosis, grade 2. ■ ● Xanthomatosis, grade 3.

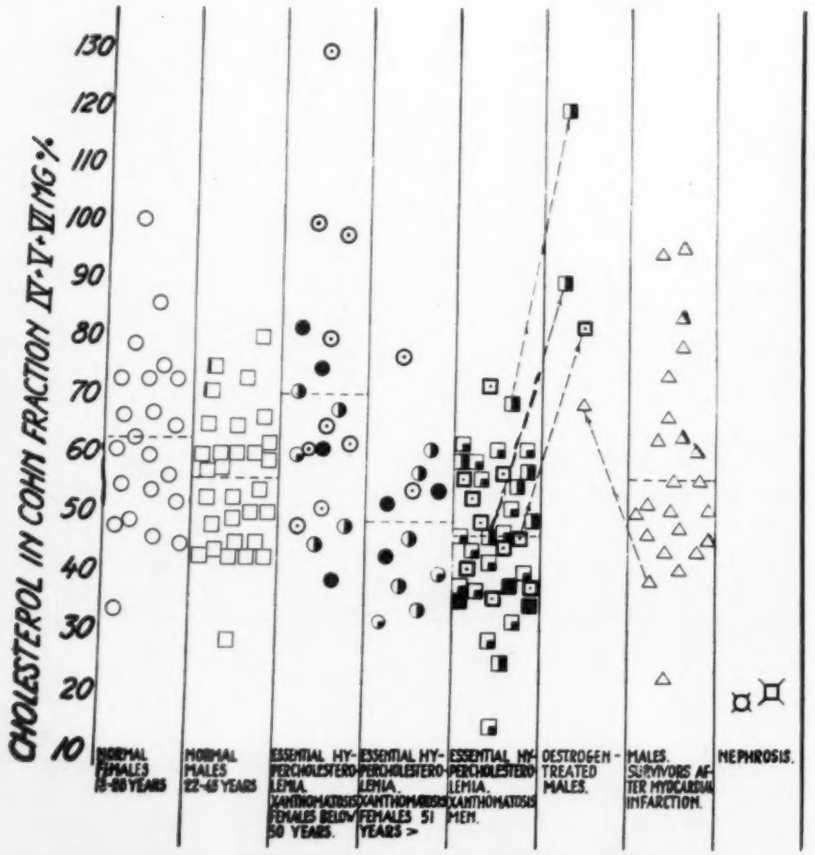

CHOLESTEROL IN COHN FRACTION IV+V+VI MG %
NORMAL FEMALES 18-80 YEARS
NORMAL MALES 22-45 YEARS
ESSENTIAL HYPERCHOLESTEROLEMIA. XANTHOMATOSIS FEMALES BELOW 50 YEARS.
ESSENTIAL HYPERCHOLESTEROLEMIA. XANTHOMATOSIS FEMALES 51 YEARS >
ESSENTIAL HYPERCHOLESTEROLEMIA. XANTHOMATOSIS MEN.
OESTROGEN-TREATED MALES.
MALES. SURVIVORS AFTER MYOCARDIAL INFARCTION.
NEPHROSIS.

FIG. 3A. Cholesterol in fraction IV + V + VI in various clinical groups.

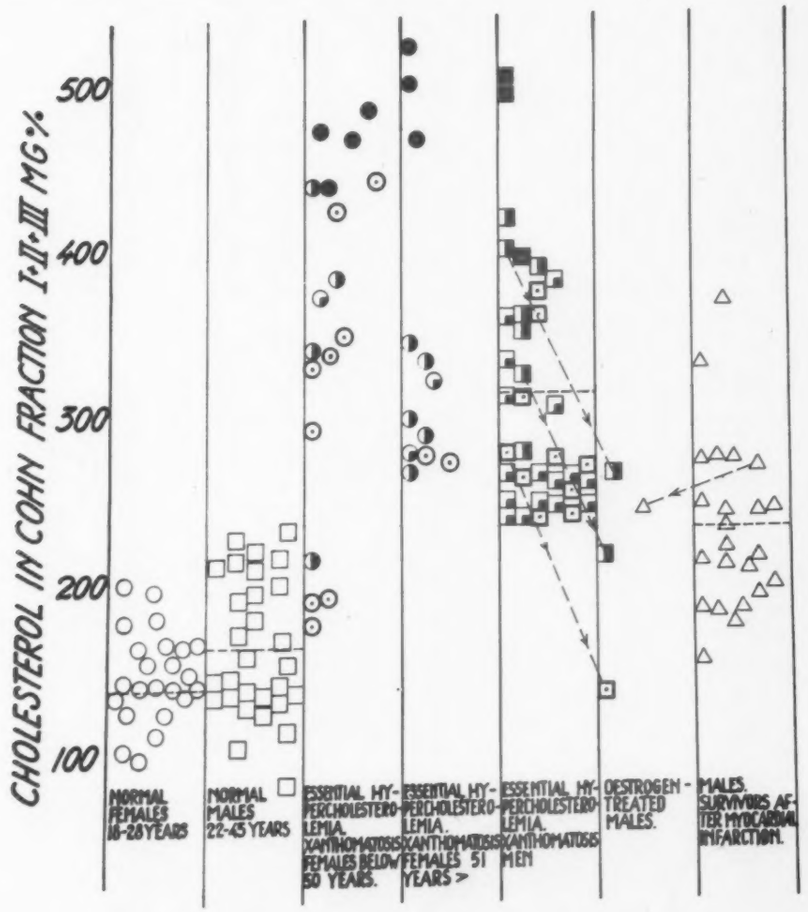


FIG. 3B. Cholesterol in fraction I + II + III in various clinical groups.

with high levels of serum cholesterol may exist with a normal concentration in this fraction.

In Figure 2B it is seen that even the most severe degree of xanthomatosis in women may have a cholesterol concentration in fraction IV + V + VI at the uppermost border of the normal range. The average cholesterol concentration in fraction IV + V + VI is not low and some of the figures in the case of essential hypercholesterolemia seem actually to be higher than normal. Two of these observations in the right lower part of the graph were made in sisters and these observations, like many of the others, represent the averaged results of three separate double determinations.

Russ et al. [*14*] have reported the occurrence of abnormal α-lipoproteins with an unusually high level of cholesterol as well as of phospholipid in fraction VI in biliary xanthomatosis. One might ask whether the high levels in some of the women with hypercholesterolemia might be due to a rise of the cholesterol concentration in this fraction [VI]. We therefore made a comparison between the cholesterol concentrations in fraction IV + V and in fraction IV + V + VI in the same plasmas. As shown in Table IV the difference is consistently small, including the findings in the subjects under discussion, A. M. K., A. L. K., E. O. and I. T.

Figure 3A gives the absolute quantity of cholesterol in fraction IV + V + VI in most of the groups fractionated. There is a striking difference between the women above fifty years of age and those below this age. The sharp increase in cholesterol in this fraction which may be caused by a large dose of oestrogen in both xanthomatosis and myocardial infarction in the male is clearly seen.

Figure 3B demonstrates the continuous transition in the cholesterol concentration in fraction I + II + III from the high levels in extensive xanthomatosis to the normal. Levels in xan-

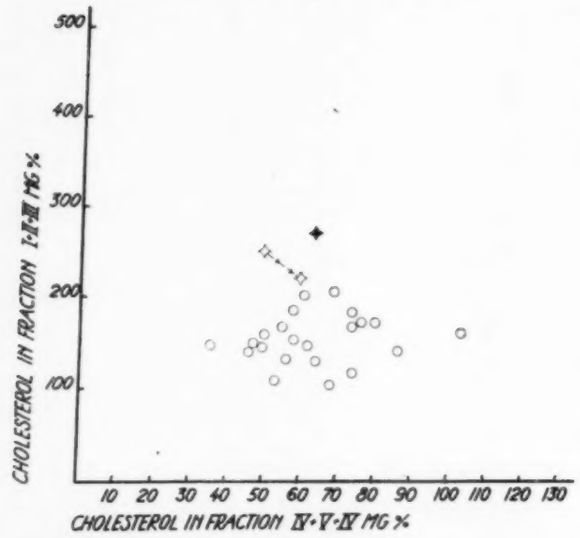


FIG. 4. Cholesterol in fraction I + II + III in two cases of congenital adrenal hyperplasia. ✦ Twenty-four year old patient with marked virilism, high 17-ketosteroid excretion. ✧ Twenty-five year old patient with well developed musculature, marked hirsutism, moderately elevated 17-ketosteroid excretion. (Each symbol represents average of duplicate determinations at two separate dates. Arrow denotes the effect of cortisone, 75 mg. administered daily for fourteen days.) ○ Normal young women, aged eighteen to twenty-eight.

thomatosis grade 1 lie within the upper range of survivors of myocardial infarction. The lower range in myocardial infarction overlaps the upper range of the normal subjects studied.

It does appear that increased production of adrenal androgenic steroids in young women may produce a level of cholesterol in Cohn fraction I + II + III definitely above the one found in the control young women.

COMMENTS

In our cases of xanthomatosis there is a continuous transition from severe cases with extensive deposits and extremely high levels of serum cholesterol to instances with moderate hypercholesterolemia and small discrete isolated nodules.

From our results it would appear that a markedly elevated level of serum cholesterol in young people is associated with the appearance of xanthomas in two to three decades only in isolated instances. On the other hand, a moderate hypercholesterolemia is associated now and then with small xanthomas after the age of forty-five to fifty years. Mann and Andrus [*15*] succeeded in producing xanthomas in a period of three years in a rhesus monkey by effecting an extreme elevation of serum cholesterol. The importance of the time factor may be relevant to the repeated comments in the literature on the difficulty or impossibility of causing a regression in tendinous xanthomas. This has been apparent even when the blood cholesterol levels have been definitely lowered for a rather long time.

We have observed a definite regression of tendinous xanthomas in one case, a fifty-two year old man who had had four attacks of myocardial infarction in less than two years. He had a rather marked elevation of β-bound cholesterol, a slight increase in the serum content of total fat, a clear serum, and symmetric xanthomas which in the Achilles tendons were somewhat larger than a big hazel nut. On a combined regimen of fat-poor diet, administration of heparin intravenously, 100 mg. twice a week, and 1.0 mg. ethinyl estradiol daily there was a marked decrease in the cholesterol concentration in fraction I + II + III. This regimen was maintained for thirteen months, after which the oestrogen was discontinued. At the end of this period the small xanthomas in the dorsal tendons of the hands had disappeared and those in the Achilles tendons could not be palpated. Continued administration of heparin and the addition of 70 to 100 gm. of corn oil daily (maximal tolerable amount) to the fat-poor diet have failed to prevent some rise in the serum cholesterol level. The Achilles xanthomas have again started to grow and at the time of writing were easily palpable and somewhat tender. There have been no further signs of myocardial infarction during the last two and a half years, his angina pectoris is much improved and the patient is at full work.

We lean towards the interpretation that xanthomatosis is not a condition apart from other states of hypercholesterolemia. Given sufficient time, small nodules may develop in moderate hypercholesterolemia.

As for the distribution of cholesterol between plasma fractions our results agree with those of other investigators to the extent that there is some average decrease in the level of cholesterol in Cohn fraction IV + V + VI in the man and woman above fifty years of age. Analysis of our results in women below the age of fifty disclosed the fact that there was a small average increase in the cholesterol concentration in fraction IV +

V + VI in this group. Even extreme xanthomatosis is compatible with high concentrations of cholesterol in fraction IV + V + VI in younger females. In this respect our data do not agree with the finding of Lever and co-workers of a distinctly low α-cholesterol in four females in the reproduction period. The high concentration of cholesterol in fraction IV + V + VI in our cases might have its explanation in a high secretion of oestrogens. We have, in agreement with others, found a marked increase in the cholesterol concentration in fraction IV + V + VI (α-cholesterol) in males with hypercholesterolemia given large doses of oestrogen.

There is general agreement that a constant feature of patients with early atheromatosis as a group is the increased β-lipoprotein level. Barr et al. [*1*] produced evidence that the cholesterol/phospholipid ratio in fraction I + III was exactly the same in patients with atherosclerosis as in normal subjects. This fact and the low average cholesterol concentration in fraction IV + V + VI (and/or α-lipoprotein) found by several groups are not readily explained by a simple hypothesis of a surplus of cholesterol as the key factor in atherogenesis.

SUMMARY AND CONCLUSIONS

1. In a large number of cases of essential hypercholesterolemia and xanthomatosis a continuous transition was observed from patients with very high cholesterol concentrations in fraction I + II + III and extensive xanthomatosis, to those with a modest increase in cholesterol in fraction I + II + III and isolated small tendinous xanthomas, to patients in whom it is impossible to state clinically whether xanthomas are present or not. This latter group merges into the ordinary group of patients with proved myocardial infarction.

2. For the production of tendinous xanthomas the time factor seems to be of great importance, as well as the concentration of cholesterol in fraction I + II + III.

3. In essential hypercholesterolemia and xanthomatosis the average concentration of cholesterol in fraction IV + V + VI is below normal in women *above* fifty years of age, and in men. In women *below* the age of fifty, on the other hand, the average cholesterol concentration in fraction IV + V + VI is somewhat greater than normal. The syndrome of severe xanthomatosis may appear in women under fifty years of age with a high concentration of cholesterol in fraction IV + V + VI.

In two young women with congenital adrenal hyperplasia there was a high concentration of cholesterol in fraction I + II + III, associated with a normal level in fraction IV + V + VI.

Acknowledgment: Our thanks are due to Dr. Welin who initiated and stimulated our interest in this field and to him and Drs. K. and G. Cramér for supplying data on their patients.

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Familial Hypercholesterolemia, Xanthomatosis and Coronary Heart Disease*

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Ann Arbor, Michigan

An association between elevated serum cholesterol levels and coronary heart disease has been recognized for a number of years [*1,2*]. There is increasing awareness, however, of the complexity of this relationship, as is brought out by the present study, part of a wider epidemiological search for situations leading to susceptibility or associated with immunity to coronary heart disease.

Families with hereditary hypercholesterolemia provide one starting point for study of susceptible groups. The presence of hypercholesterolemia in the families which are the subject of the present communication came to attention in 1929 when Curtis, Wile and Eckstein reported two sisters with xanthomatosis [*3*]. A systematic survey of the entire family led Wilkinson and his co-workers to the conclusion that hypercholesterolemia was due to the presence of a single, incompletely dominant gene while xanthomatosis represented a condition characterized by the coexistence of two such alleles [*4*], a conclusion also reached by Adlersberg, Parets and Boas [*5*]. Wilkinson and his group, however, contended that coronary disease was not unduly prevalent among the heterozygous carriers of the abnormal gene [*6*], a statement lately quoted again by Ahrens and associates [*7*]. The present report is based on a study of the same sibships. The relationship between abnormal cholesterol metabolism and coronary heart disease will again be considered. The mode of inheritance of hypercholesterolemia will be analyzed and evaluated in the light of recent studies [*8–11*] which specifically take issue with the earlier findings of Wilkinson and associates [*4*].

METHODS OF STUDY

The participants were examined at home; roentgenograms were obtained at a local hospital. Medical histories were taken by one of us (F. H. E.), assisted by a public health nurse or a senior medical student, and included a detailed record of illness in the respondents and their relatives, as well as questions on social factors and personal habits, not currently analyzed. Each person was interviewed individually and family histories were thus cross-checked. A total of 268 records were collected. In 237 instances the records included a complete history and a thorough physical examination; a blood sample was obtained in all but seventeen of these 237 subjects. Thirty-one persons had a brief history and blood test only. With the exception of that group and the babies, electrocardiograms were recorded in all participants (12-lead tracings on those above their mid-teens; standard and unipolar limb leads on children). Six foot roentgenograms of the chest were taken of persons aged twenty-five and over. Lateral roentgenograms of the abdomen, for the visualization of aortic calcifications, were taken for initial screening but without intention to follow up this phase, in view of the possible radiological hazard involved [*12*]. Blood samples were obtained non-fasting, immediately spun down and kept unfrozen in the cold for no more than five days until analyzed for total

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† Special Research Fellow, National Heart Institute, U. S. Public Health Service.

serum cholesterol by the method of Abell and associates [*13*]. The urine of almost all participants was examined for albumin and sugar.

DESCRIPTION OF THE SIBSHIPS

The families are centered around the two large sibships B and C. (Fig. 1, Table I.) Sibships and individuals have been numbered as in the earlier study [*4*] but their sequence has been rearranged by age. The parents of both sibships B and C came to this country from Alsace-Lorraine, initially settled as farmers in Illinois, and moved to Michigan about thirty-five years ago; with the exception of G1 and E2, all participants were born in the United States. Most belong to a small religious community. The families are closely knit but there has been no intermarriage of blood relatives at any point. Most of the older men were originally farmers but for the past ten or twenty years frequently carried on other work as well, such as bricklaying, carpentry or trucking: their physical activity was thus generally heavy. About a third of the younger men similarly combined farming with other jobs; the remainder were employed largely in industry although most of them lived in rural or semi-rural areas. Most of the families were in comfortable economic circumstances.

All subjects studied ten years ago have been reexamined, with the following exceptions: A14, B12, BJ3, C11x, BK1 and E3 died in the interval; B15, B16, DF1, DK1, B3, B13, B14, C8x and CB2 were uncooperative, and CC4, CF1, BJ9, BJ11, DG1, G4 and FC1 moved away or were not traced. Altogether, 268 examinations were made, as compared with 159 examinations in the previous survey, the difference being accounted for by persons not previously examined and younger children.

SERUM CHOLESTEROL LEVELS

In previous studies dealing with the mode of inheritance of "hypercholesterolemia," the first step consisted in establishing a serum cholesterol level which would separate "hypercholesterolemic" from "normocholesterolemic" subjects. This division assumes that the cholesterol level chosen does, in fact, allow a distinction between two genetically different populations. If a single, dominant gene of considerable penetrance were involved, as has been generally believed, a bi-modal frequency distribution of serum cholesterol values would be expected, with a trough in the region of the cholesterol level indicative of the best line of demarcation between the two populations. The demonstration of bi-modality in frequency distribution would yield the most objective evidence for the presence of such a gene.

Among the families currently studied, the age adjusted frequency distribution curve suggested that there might be bi-modality among the men but not among the women. (Fig. 2.) In plotting these curves, each individual serum cholesterol value was adjusted to its presumed level at age twenty. Age adjustments were necessary since mean serum cholesterol levels of population groups are age dependent, as originally shown by Keys et al. [*14*] and largely confirmed since [*15,16,21*]. It would have been preferable to plot frequency distribution curves by age groups, testing each curve thus obtained for bi-modality, since age adjustment for each individual value requires the unwarranted assumption that serum cholesterol levels change with age in each member of the population to the same extent. Unfortunately, the sample was too small for plotting age specific frequency distributions. The data were therefore age adjusted and pooled. For age corrections, the data from the cooperative study on lipoproteins [*15*] were used, even though it is admittedly undesirable to transpose findings of this kind from one population group to another. These data provide mean serum cholesterol levels by age, beginning at age twenty. It was assumed, as suggested by the data of Adlersberg, Schaefer and Steinberg [*16*], that there is no significant age trend prior to that age. For instance, according to the cooperative study, the mean serum cholesterol level among men was 199 mg. per cent between the ages of twenty and twenty-four, and 213 mg. per cent between the ages of twenty-five and twenty-nine; consequently, the age adjusted level of a man, aged twenty-eight, with an actual level of 230 mg. per cent, would be 216 mg. per cent.

In interpreting these frequency distribution curves the variability of serum cholesterol values must be kept in mind. Each individual serum cholesterol determination is subject to three sources of variation: the technical error of duplicates run simultaneously, the technical error of replicates run at different times, and the biological fluctuations in level. A single cholesterol measurement will, therefore, characterize a

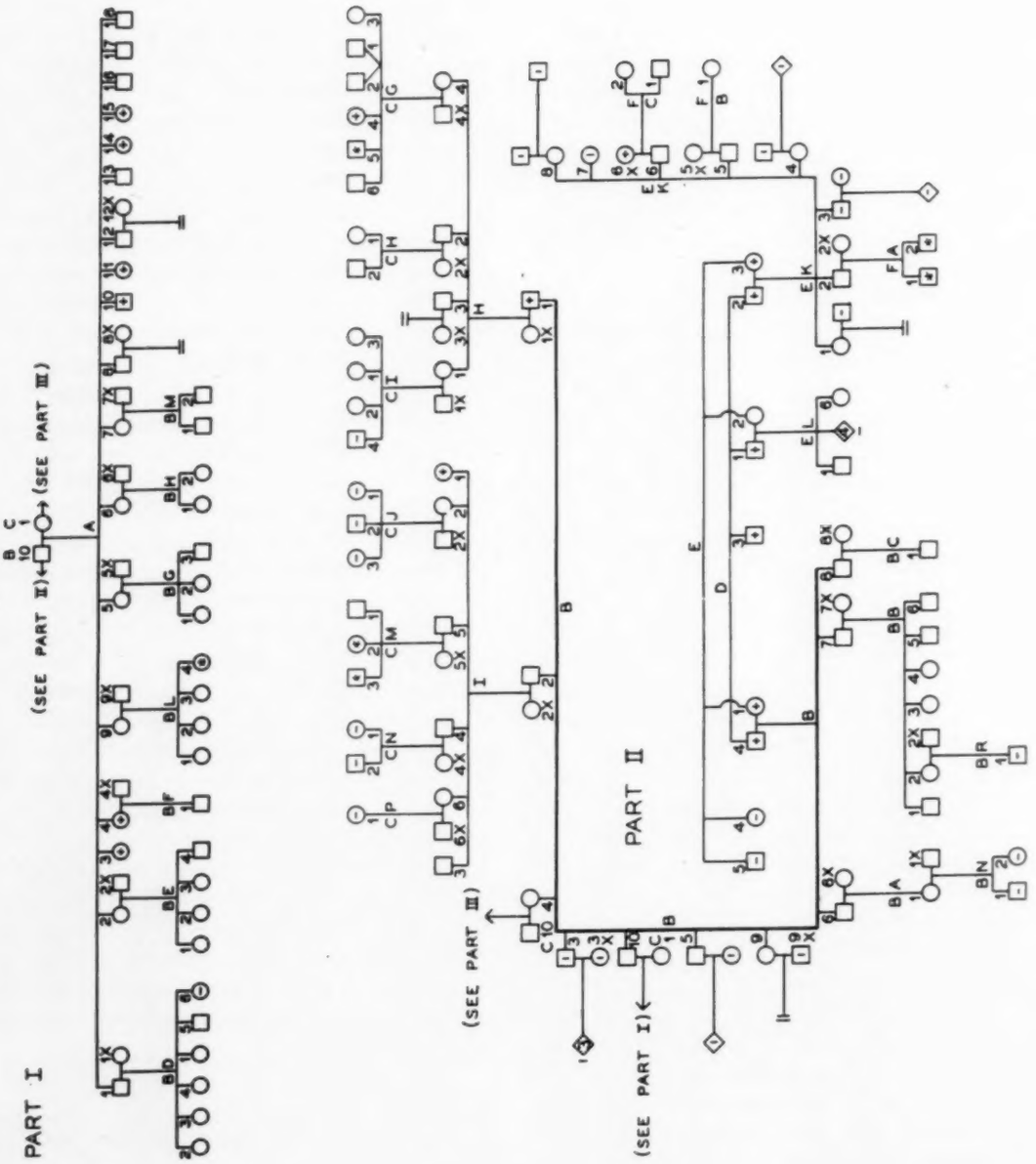
person with no more than a limited margin of confidence. These uncertainties surrounding a single value would generally tend to obscure a bi-modality in frequency distribution even if the curve did, in fact, represent a mixture of two different populations. The conclusion is nevertheless drawn that the frequency curves provide no clear-cut evidence for the existence of a dividing point between "hypercholesterolemic" and "normocholesterolemic" persons. It is not, therefore, strictly justifiable to mark subjects with symbols indicating "hypercholesterolemia" or "normocholesterolemia," even though this would have made it easier to see the genetic interrelationships between these subjects at a glance. With these misgivings in mind, the data may, however, be analyzed in the conventional way, using a serum cholesterol level of 280 mg. per cent as a dividing point. By this standard there were fourteen matings between a "hypercholesterolemic" person and his or her "normal" spouse; twenty-two of their fifty-eight children were found to be "hypercholesterolemic" by the same standard, suggesting that "hypercholesterolemia" might be associated with the presence of a single, incompletely dominant gene. When both parents were "hypercholesterolemic" (B10 and C1), at least fourteen of their eighteen children were "hypercholesterolemic"; xanthomatosis was observed among six of the eighteen children. These findings are similar to those reported by Wilkinson and associates ten years ago but a detailed comparison of our respective findings is not feasible in view of the wide discrepancy (70 mg. per cent) noted by the previous authors between the results of their cholesterol determinations by the Bloor and Schoenheimer-Sperry methods [*4*]. Some exceptions were noted which do not, however, vitiate these general conclusions. Thus, it might happen by chance that the first two children of a mother with hypercholesterolemia are normocholesterolemic (A7, BM1 and BM2); further offspring might be hypercholesterolemic. In the case of BK4, frankly hypercholesterolemic, two of the children, aged six and three years, had serum cholesterol levels of 243 and 236 mg. per cent, respectively; in view of the age trends, the levels may exceed 280 mg. per cent in both children as they grow older. In the case of C10, aged sixty-six, in whom the serum cholesterol level was "normal" (243 mg. per cent on the first examination and 256 mg. per cent in another specimen drawn five months later), one son had a level of 291 mg. per cent; C10, however, had a serum cholesterol level of 320 mg. per cent ten years ago. Finally, C12x, with a level of 316 mg. per cent on two independent occasions, has only normocholesterolemic children; her level was only 175 mg. per cent ten years ago but no clinical reason for the presently high level could be elicited.

The use of a fixed cutting point, 280 mg. per cent, to delineate "hypercholesterolemic" and "normocholesterolemic" persons is clearly incorrect, in view of the age dependence of serum cholesterol values. It is surprising, therefore, that no more than four men and nine women in the entire series might have been incorrectly designated as "hypercholesterolemic" by using 280 mg. per cent, regardless of age, as the dividing line. In order to arrive at this approximate estimate, the following cutting points were used: 280 mg. per cent for both sexes at ages under twenty-five, 300 mg. per cent for men and 290 mg. per cent for women at ages twenty-five to forty-four, 320 mg. per cent for men and 330 mg. per cent for women at age forty-five and over. These cholesterol values roughly represent levels beyond 2 standard deviations from the age specific population mean [*15*], assuming a standard deviation of 40 mg. per cent.

Since meaningful genetic patterns emerge when a serum cholesterol level of 280 mg. per cent or a similar age adjusted level is used as a dividing point, this might be thought to support both the concept of inheritance involving a single, dominant gene and the correctness of using these particular cholesterol levels for the detection of the carriers of this gene. Further support for such an interpretation would have been provided by demonstrating a bi-modality in frequency distribution of serum cholesterol values; the evidence for this, as mentioned, was equivocal. These problems and their relationship to the inheritance of xanthomatosis will be further discussed.

CARDIAC MANIFESTATIONS OF XANTHOMATOSIS

Xanthomatosis occurred among six (A3, A4, A10, A11, A14, A17) of the eighteen children comprising sibship A. (Fig. 1.) Only two (A14, A17) of these six siblings were alive at the time of the previous study [*4*], the others having died suddenly, presumably of coronary heart disease; in A10, although not previously designated as


PART I
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PART II
(SEE PART III)
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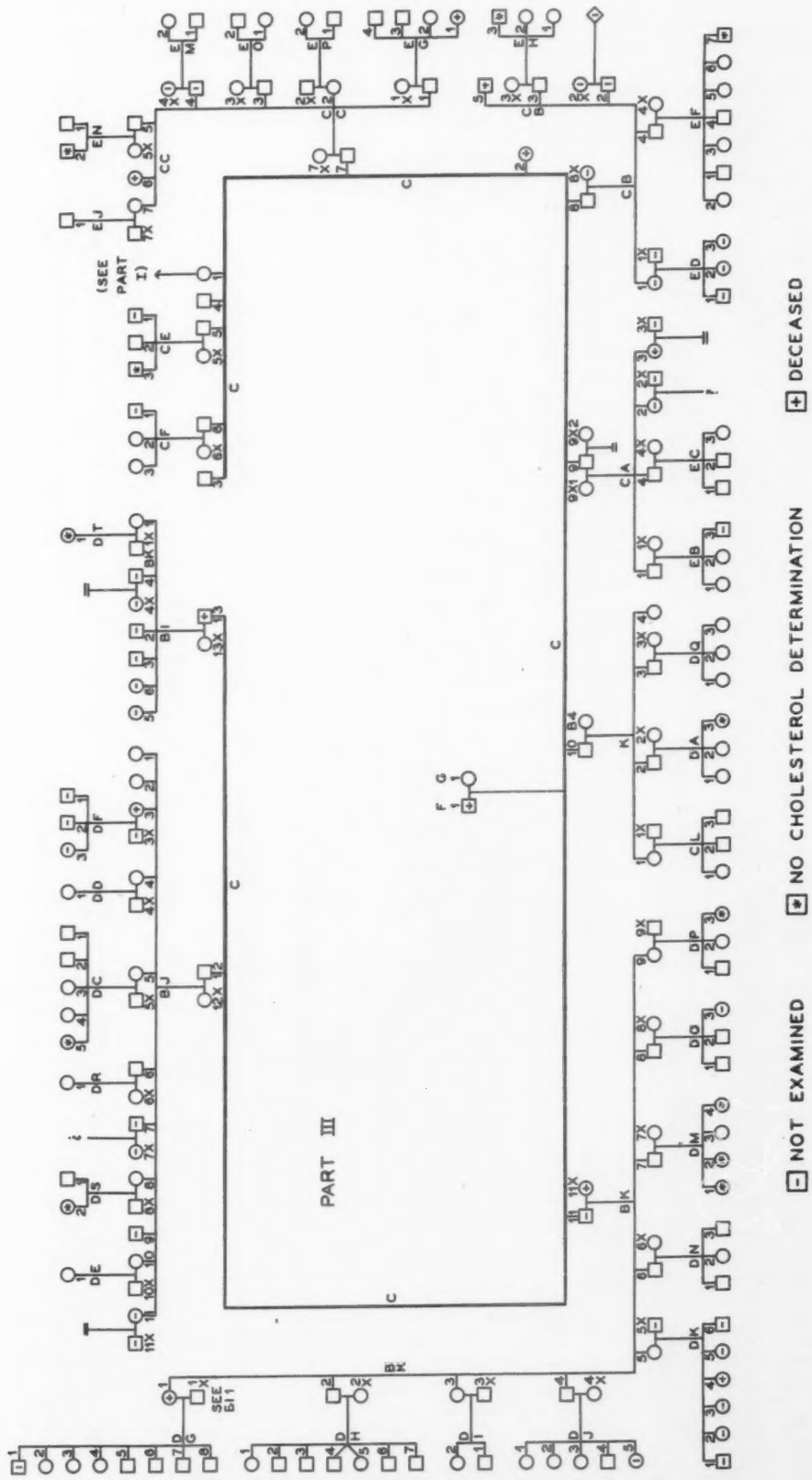

FIG. 1. (Parts I–III). Pedigree of the families studied. Sibship A (Part I) is the offspring of B10 (Part II) and C1 (Part III).

TABLE I

SERUM CHOLESTEROL VALUES (MG. %) BY SIBSHIPS AND FAMILY UNITS

Kindred No.	Age (yr.)	Cholesterol	Kindred No.	Age (yr.)	Cholesterol	Kindred No.	Age (yr.)	Cholesterol
I. Intermarriage between Sibships B and C								
B 10	58	485	BD 1	10	295	A 6	31	267
C 1	59	460	BD 2	15	272	A 6x	31	228
A 1	39	490	BD 3	13	382	BH 1	10	240
A 2	38	395	BD 4	12	271	BH 2	6	187
A 5	32	342	BD 5	6	324	.....	..	...
A 6	31	267	.....	..	...	A 7	29	445
A 7	29	445	A 2	38	395	A 7x	32	249
A 8	28	186	A 2x	41	261	BM 1	7	170
A 9	34	337	BE 1	16	172	BM 2	4	133
A 12	23	308	BE 2	13	371	.....	..	...
A 13	22	349	BE 3	9	306	A 9	34	337
A 16	17	193	BE 4	5	139	A 9x	36	246
A 17	+	1033	.....	..	...	BL 1	8	282
A 18	11	470	A 4x	45	261	BL 2	7	270
			BF 1	13	320	BL 3	3	137
A 1	39	490	.....	..	...	.....	..	...
A 1x	35	241	A 5	32	342	.....	..	...
			A 5x	36	243	.....	..	...
			BG 1	13	296	.....	..	...
			BG 2	10	137	.....	..	...
			BG 3	8	331	.....	..	...
II. Sibship B and Related Family Units								
B 2	62	262	I 2	37	248	BB 3	18	135
B 4	61	191	I 2x	38	240	BB 4	17	143
B 5	56	187	.....	..	...	BB 5	15	138
B 6	52	295	I 3	26	207	BB 6	13	155
B 7	50	195	.....	..	...	.....	..	...
B 8	47	499	I 4	34	199	B 8	47	499
B 9	54	439	I 4x	31	164	B 8x	45	184
B 10	58	485	.....	..	...	.....	..	...
			I 5	36	212	BC 1	18	464
B 1x	71	233	I 5x	27	158	.....	..	...
			CM 1	7	188	B 9	54	439
H 1	37	245	.....	..	...	.....	..	...
H 1x	39	185	I 6	31	187	E 2	84	245
CI 1	14	191	I 6x	33	216	.....	..	...
CI 2	10	185	.....	..	...	EL 1	50	237
CI 3	17	155	B 6	52	295	EL 6	50	239
			B 6x	45	250	.....	..	...
H 2	40	186	.....	..	...	EK 1	58	288
H 2x	37	164	BA 1	26	286	EK 2	54	277
CH 1	15	151	BA 1x	28	216	EK 2x	43	199
CH 2	11	169	.....	..	...	EK 4	51	282
			B 7	50	195	.....	..	...
H 3	39	400	B 7x	45	173	EK 5	45	277
H 3x	35	245	.....	..	...	EK 5x	43	229
			BB 1	24	158	FB 1	10	202
H 4	41	174	.....	..	...	.....	..	...
H 4x	40	276	BB 2	22	197	EK 6	44	318
CG 1	12	165	BB 2x	25	156	FC 1	18	148
CG 2	12	170	.....	..	...	FC 2	16	198
CG 3	14	162	.....	..	...	.....	..	...
CG 6	3	154	.....	..	...	EK 8	40	229
B 2	62	262	.....	..	...	.....	..	...
B 2x	62	215	.....	..	...	.....	..	...

TABLE 1 (*Continued*)

SERUM CHOLESTEROL VALUES (MG. %) BY SIBSHIPS AND FAMILY UNITS

Kindred No.	Age (yr.)	Cholesterol	Kindred No.	Age (yr.)	Cholesterol	Kindred No.	Age (yr.)	Cholesterol
III. Sibship C and Related Family Units								
C 1	59	460	C 9	64	218	BK 4	37	374
C 3	49	367	.....	..	...	BK 4x	32	187
C 4	57	187	CA 1	39	250	DJ 1	12	139
C 5	54	366	CA 1x	36	181	DJ 2	9	163
C 6	53	172	EB 1	13	161	DJ 3	6	243
C 7	60	199	EB 2	8	183	DJ 4	3	236
C 8	63	239	.....	..	...	.....	..	...
C 9	64	218	CA 4	37	271	BK 5	34	358
C 10	66	243	CA 4x	30	181	.....	..	...
C 12	68	190	EC 1	13	171	BK 6	33	159
			EC 2	8	198	BK 6x	31	150
C 5	54	366	EC 3	5	202	DN 1	7	143
C 5x	50	225	.....	..	...	DN 2	6	168
CE 2	10	164	C 10	66	243	DN 3	2	149
			B 4	61	191	.....	..	...
C 6	53	172	.....	..	...	BK 7	31	434
C 6x	51	227	K 1	40	236	BK 7x	27	166
CF 2	14	119	K 1x	44	257	DM 3	5	180
CF 3	12	159	CL 1	20	160	.....	..	...
			CL 2	16	127	BK 8	30	194
C 7	60	199	CL 3	6	156	BK 8x	28	189
C 7x	59	279	.....	..	...	DO 1	6	219
			K 2	39	291	DO 2	4	245
CC 1	38	234	K 2x	35	210	.....	..	...
CC 1x	35	226	DA 1	11	180	BK 9	28	195
EG 2	7	209	DA 2	4	213	BK 9x	32	182
EG 3	6	236	.....	..	...	DP 1	7	154
EG 4	2	202	K 3	35	256	.....	..	...
			K 3x	32	243	DP 2	5	205
CC 2	35	201	DQ 1	9	228	.....	..	...
CC 2x	31	195	DQ 2	6	255	C 12	68	190
EP 1	8	166	DQ 3	3	187	C 12x	67	316
EP 2	4	156	.....	..	...	.....	..	...
			K 4	29	238	BJ 1	43	188
CC 3	33	204	.....	..	...	BJ 2	41	183
CC 3x	29	209	BK 1x	43	241	.....	..	...
EO 1	3	164	DG 2	17	171	BJ 4	38	160
EO 2	22 m	167	DG 3	16	160	BJ 4x	39	307
			DG 4	14	294	DD 1	5	155
EM 1	9	203	DG 5	12	165	.....	..	...
EM 2	6	207	DG 6	10	341	BJ 5	37	169
			DG 7	8	360	BJ 5x	39	310
CC 5	29	188	DG 8	6	179	DC 1	9	185
CC 5x	27	171	.....	..	...	DC 2	7	146
EN 1	4	165	BK 2	41	247	DC 3	6	201
			BK 2x	35	167	DC 4	4	169
CC 7	19	272	DH 1	17	188	.....	..	...
CC 7x	21	183	DH 2	16	165	BJ 6	35	237
EJ 1	18 m	154	DH 3	13	153	BJ 6x	32	183
			DH 4	8	198	DR 1	9	185
C 8	63	239	DH 5	8	162	.....	..	...
			DH 6	4	134	BJ 8	32	221
CB 3	35	210	DH 7	2	108	BJ 8x	35	206
CB 3x	31	172	.....	..	...	DS 1	7	164
EH 1	5	141	BK 3	39	444	.....	..	...
EH 2	3	139	BK 3x	42	232	.....	..	...
			DI 1	11	144	BJ 10	27	189
CB 4	38	214	DI 2	12	218	BJ 10x	29	227
CB 4x	33	169	.....	..	...	DE 1	1	146
EF 1	12	154	.....	..	...	.....	..	...
EF 2	15	151	.....	..	...	C 13x	69	226
EF 3	8	253	.....	..	...	.....	..	...
EF 4	3	216	.....	..	...	BI 1	41	165
EF 5	3	163	.....	..	...	BK 1x	See	BK1 ...
EF 6	2	188	.....	..	...	.....	..	...

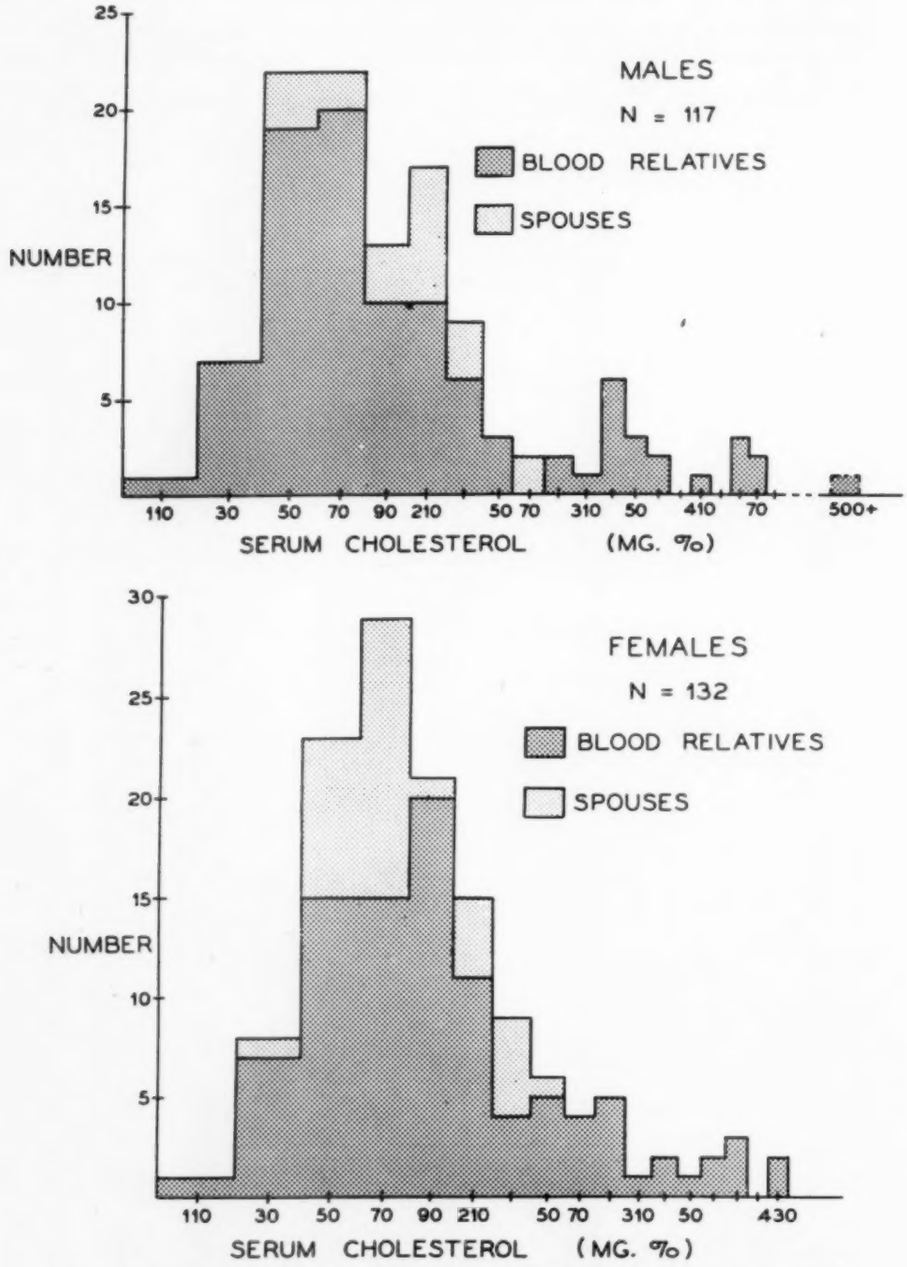

FIG. 2. Frequency distribution of age-adjusted serum cholesterol values by sex.

being affected with xanthoma, typical skin lesions were present. The remaining two youngest children (A14, A17) have since died; their history and autopsy findings will be reported separately [*17*]. All these children showed xanthomas early in life; according to their mother, all had unmistakable lesions by the age of four. Their ages at death, in the order of their births, were, respectively, thirteen, twenty-three, seven, fifteen, sixteen and thirteen years. There is no doubt that the disorder associated with xanthomatosis is a uniformly fatal disease when it occurs at an early age in this family. It is possible that persons in whom xanthomas develop later in life are the ones who often live into middle age and beyond.

TABLE II
CORONARY HEART DISEASE

Family Number	Age (yr.)	Sex	Weight (lb.)	Height (inches)	"Heart Attack"	Duration of Angina Pectoris (yr.)	Blood Pressure (mm. Hg)	Electrocardiogram	Serum Cholesterol (mg. %)
C 11	67	M	175	68	1956 (mild)	8	140/70	Flat T waves	331
C 5	54	M	172	66	None	6	120/70	Normal	366
B 8	47	M	178	70	None	2	155/90	Normal	499
C 8	63	F	...	..	1956	15	170/85	Q_3 T_3 pattern with deep Q_aVf	239

CARDIAC MANIFESTATIONS AMONG PERSONS WITHOUT XANTHOMATOSIS

During the earlier survey, except for the children with xanthomatosis only one man (C12), who was normocholesterolemic and fifty-nine years old at the time, gave evidence of questionable coronary heart disease [*4*]; he continues to have atypical chest pain which is not characteristic of angina pectoris and his electrocardiogram has remained normal. In the course of the intervening ten years in three previously healthy men clinical evidence of coronary heart disease developed, based on typical symptoms of angina pectoris; all are hypercholesterolemic. (Table II.) There were no deaths from heart disease among the men since the previous study but one additional man (B1) who was presumably hypercholesterolemic since one of his children but not his wife has an elevated serum cholesterol level, had already died suddenly, presumably of a coronary attack, prior to the earlier survey.

Among the women, manifest coronary heart disease appeared to be relatively less common than among the men. Only one woman, belonging to sibship C but normocholesterolemic (C8), experienced an attack of myocardial infarction, at the age of sixty-two; her present electrocardiogram was suggestive but not diagnostic of an old, posterior wall infarct. (Table II.) One woman (C11x), who married into sibship C, died of "cardiorenal disease and dropsy" at the age of sixty-four; she was stated to be hypertensive and her serum cholesterol level was normal ten years ago. Another woman (G1) the mother of sibship C, died in 1957 at the age of ninety, following an acute infarction of the large bowel On necropsy, performed by Dr. W. A. DeYoung, the heart was of normal weight and the coronary vessels were widely patent, showing no more than a moderate amount of sclerosis. Her husband (F1) who had died prior to the earlier survey, may be presumed to have transmitted the hypercholesterolemic trait to approximately half of his children, his wife being normocholesterolemic now, as she was ten years ago; he apparently suffered from angina pectoris since his son (C11) volunteered the statement that his father experienced pain identical to his own.

HYPERTENSION

Since blood pressure levels very probably represent a continuous variable [*18*], it is neither desirable nor correct to demarcate fixed upper limits for "normal" pressures. In view of the small size of the present series and the difficulty in finding a comparable reference population, it was decided to designate diastolic blood pressures over 90 mm. Hg, irrespective of age, as in the "upper range"; pressures were read to the nearest 5 mm. For the comparison of blood pressure and serum cholesterol levels, the latter were assigned to the "upper range" at levels of 280 mg. per cent and above, irrespective of age. While these definitions are arbitrary, they permit approximate estimates of the prevalence of "hypertension" and "hypercholesterolemia" and their interrelation among these families. (Table III.)

Among fifteen men aged forty-five years and over, blood pressures in the upper range were seen in four instances; at ages twenty-five to

TABLE III

INTERRELATIONSHIPS BETWEEN BLOOD PRESSURE AND SERUM CHOLESTEROL RANGES*

Blood Pressure	Age 25–44			Age 45 and Over		
	Serum Cholesterol					
	Upper Range	Lower Range	Total No.	Upper Range	Lower Range	Total No.
Men						
Upper Range	1	3	4	2	2	4
Lower Range	6	31	37	4	8	12
Total No.	7	34	41	6	10	16
Women						
Upper Range	0	38	38	1	2	3
Lower Range	7	1	8	3	11	14
Total No.	7	39	46	4	13	17

* For definition of ranges: see text.

forty-four years, such levels were found among four of forty-two men. Of a total of seventeen women aged forty-five years and over, three showed elevations of blood pressure as herein defined; in only one of forty-six women aged twenty-five to forty-four years did levels fall into the upper range. Diastolic pressures above 90 mm. Hg were not found among any of the persons under the age of twenty-five in either sex.

It would be pointless, because of the inadequate number of observations, to subject these findings to tests of statistical significance. However, if a rough comparison with blood pressure readings on large, more or less random population groups [*18–20*] is made, it would seem that hypertension was not unduly frequent in these families. There was also no obvious association between blood pressure and serum cholesterol levels, in accordance with the experience of others [*20*].

MISCELLANEOUS FINDINGS

Roentgenograms of the chest and lateral views of the abdomen were obtained in forty-five men and forty-six women under the age of forty-five; none of these showed cardiac enlargement or aortic calcifications. Of fourteen men aged forty-five years and over, in thirteen the cardiac size was normal and in one it was of borderline dimensions. In seven of these men, however, there were calcifications in the aorta; in two instances these were located in the aortic arch, in the remaining five men such lesions were present in the abdominal aorta. None of these men had diastolic blood pressures above 90 mm. Hg; the numbers involved were insufficient to relate serum cholesterol levels to aortic calcifications. Of fourteen women aged forty-five and over, the heart was of normal size in all but one, with borderline enlargement; in seven of these fourteen women there were calcifications in the abdominal aorta and corresponding lesions in the arch were concomitantly present in two. Three of these women were unequivocally hypertensive. Thus, about half of these older men and women in this limited series showed roentgenographic evidence of rather advanced aortic atheromatosis, a figure somewhat higher than observed in a random population of elderly persons [*21*].

The previous investigators observed only one instance of corneal arcus (G1) in this group of families [*4*]. Three additional instances were

discovered at the present time (B10, A17 and B9); all were hypercholesterolemic. Xanthelasma was found in four hypercholesterolemic women (C1, C4x, B9 and BK3) and one normocholesterolemic woman (BJ5); a questionable lesion was noted in G1 and also in BK9 in whom the serum cholesterol level was normal although her sister (BK3) had both xanthelasma and hypercholesterolemia.

Another interesting familial disturbance was encountered: DG4, aged fourteen, and her brother DG7, aged eight, both showed a partial heart block in the electrocardiogram; none of the other siblings was similarly affected. Both tracings were repeated a month later and the findings were confirmed. Another child (DN2) has congenital heart disease, probably a ventricular septal defect; her brother (DN1) has hypospadias and her mother (BK6x) has a pronounced squint; this familial concentration of defects may be more than coincidental. A search for other familial aggregations of disease was unrewarding. A few of the women had thyroid enlargement or had had thyroidectomy; the serum cholesterol levels were well within normal range in all of these and none had clinical signs of thyroid dysfunction. Urine examinations revealed one instance of diabetes (FB2) but no renal abnormalities of significance.

Some of the participants had isolated electrocardiographic findings of possibly marginal significance. Spontaneous tachycardia and borderline S-T depressions were observed in I4, aged thirty-four, who gave no history of cardiovascular disease in his parents or siblings and had normal serum cholesterol and blood pressure levels. Very tall T waves in leads V_2 to V_5 occurred in BK2; all other findings in this man were normal but his father has coronary heart disease. Two men (BK3x and A7x) not belonging to the two main kindreds, had a complete right bundle branch block and an early left ventricular strain pattern, respectively. One woman (CB4x), aged thirty-three, had an isoelectric T_2 wave and T wave inversions in V_1 to V_5, as occasionally observed in younger persons in the apparent absence of cardiac disease.

COMMENTS

Two aspects of the present study deserve detailed comment: the mode of inheritance of "hypercholesterolemia" and the relation between coronary heart disease and the serum cholesterol level. With regard to the first of these questions, the genetic model proposed by Wilkinson and his group [*4*] and by Adlersberg, Parets and Boas [*5*] had, until recently, found wide acceptance, suggesting that there is but one dominant gene involved in this metabolic disturbance. Singly, this gene would be responsible for the "forme fruste" of xanthomatosis, i.e., familial hypercholesterolemia, while two alleles of this gene would lead to more markedly elevated serum cholesterol levels and to xanthomastosis. Recent critics of this theory have claimed that homozygosis is not a prerequisite of xanthomatosis since a person with this condition may have a normocholesterolemic parent or a normocholesterolemic child [*8–11*]. According to this view, xanthomatosis reflects the presence of a single, dominant gene. Wheeler's data fully support this theory [*11*]. Piper and Orrild's data lend at least partial support inasmuch as almost all children with xanthomatosis in their series had a xanthomatous parent, irrespective of the serum cholesterol level in the collateral parent [*8*].

While it has become difficult to equate xanthomatosis with homozygosity, the alternative theory also is open to criticism. In particular, the occurrence of normocholesterolemia in the children of xanthomatous persons seems to be the exception rather than the rule, except in Wheeler's series [*11*]. Harris-Jones and coworkers quote only one such instance in which the son of a xanthomatous father had a cholesterol level of 200 mg. per cent [*10*]. Leonard [*9*] stated that twelve children with normal cholesterol levels were born to parents with xanthomatosis but the actual values were not given except in two instances in which levels of 256 and 263 mg. per cent were considered normal, the age of these children not being stated. In order to pursue this analysis further, Piper and Orrild's data [*8*] have been examined in detail to determine the frequency of "hypercholesterolemia" and "normocholesterolemia" in the offspring of a xanthomatous parent and his or her non-xanthomatous spouse. The issue of thirty-nine matings of this type included ninety-four children with recent serum cholesterol determinations. (Table IV.) Eighty-two per cent of these children had elevated serum cholesterol levels, using 280 mg. per cent as the dividing point; the vast majority of these showed levels above 325 mg. per cent. It is concluded that the frequency of hypercholesterolemia among the

TABLE IV

SERUM CHOLESTEROL LEVELS AMONG CHILDREN OF ONE XANTHOMATOUS AND ONE NON-XANTHOMATOUS PARENT, BASED ON DATA BY PIPER AND ORRILD [8]

Serum Cholesterol Level in Non-Xanthomatous Spouse (mg. %)	No. of Matings	No. of Children			
		Total	Serum Cholesterol (mg. %)		
			325 and Over	280–324	Less than 280
335–362	6	17	13	2	2
199–290	9	32	27	1	4
Unknown	24	45	29	5	11
Total	39	94 (100%)	69 (73%)	8 (9%)	17 (18%)

issue of such matings is much higher than would be expected if the xanthomatous parent carried only one abnormal, dominant gene, transmittible to about half of his or her children. These findings would be more consistent with the hypothesis that persons with xanthomatosis usually carry two abnormal genes. The true frequency of hypercholesterolemia among these children in Piper and Orrild's series is probably even higher than 82 per cent. In our own experience, in members at ages below twenty-five years levels in excess of 245 mg. per cent must be suspected of being in the high range; in the present series, all but two children in this age group showed levels below 245 mg. per cent unless at least one of their parents was unequivocally hypercholesterolemic. Using the latter dividing point, the number of normocholesterolemic children in Piper and Orrild's series would be reduced from seventeen to eleven and the frequency of hypercholesterolemia would become 88 instead of 82 per cent, making the likely assumption that most of these children were less than twenty-five years old.

If persons with xanthomatosis were, in fact, usually homozygous for the abnormal gene, the mating of two hypercholesterolemic, non-xanthomatous persons would be expected to result in xanthomatosis among approximately 25 per cent of their children. Considering the crucial importance of this matter, it is surprising that the literature contains, to the best of our knowledge, only four fully documented instances of matings between two hypercholesterolemic, non-xanthomatous persons; in all others the parents were presumed but not proved to be heterozygous. These four instances are represented by the union of B10 and C1 in the current series, two matings reported by Adlersberg, Parets and Boas (Figs. 4 and 5 [5]); and the marriage between two first cousins depicted in Piper and Orrild's pedigree II [8]. It would seem tenuous to base a general genetic theory upon such limited supporting data. It must be concluded that the available evidence provides no firm ground for attributing xanthomatosis to the presence of either a single gene, as already discussed, or, alternatively, to the coexistence of two abnormal alleles.

In order to be fully convincing, any genetic model of xanthomatosis must link the latter condition with its less severe variant, hypercholesterolemia. Piper and Orrild [8] correctly state that these two conditions form a continuous chain of events, the difference in the clinical manifestations being merely a reflection of the degree of elevation of serum cholesterol. These authors fail, however, to provide a reason why serum cholesterol levels may vary so markedly among the carriers of the supposedly single, abnormal gene. The theory which equated homozygosity with xanthomatosis provided such an explanation, but, as mentioned before, cannot be fully supported on current evidence.

Even its corollary, the equation of heterozygosity with more moderate degrees of hypercholesterolemia, is open to question since, as was shown earlier, frequency distributions of serum cholesterol levels are not unequivocally bi-modal, although the variability of single serum cholesterol measurements, as previously mentioned, might tend to obscure a true bi-modality of distribution. Evidence for the presence of a single gene, in terms of bi-modality in frequency distribution, might also be concealed if this gene were under the moderating influence of multiple environmental and host factors; under such conditions the expression of this gene could be evaluated only against the background of the entire epidemiologic or, more broadly, ecologic setting of the persons under study. In this light, the concept of "essential hypercholesterolemia" might eventually become so blurred as to resemble the situation which Pickering has found to exist in the case of "essential hypertension," a concept, in fact, without adequate foundation [*22*]. This interpretation would seem to be akin to the more recent views of Adlersberg and associates, published in abstract form [*23*]. Wilkinson has also found it necessary to modify his earlier hypothesis, suggesting that the inconsistencies inherent in current genetic models could be bridged by postulating the existence of multiple alleles of the abnormal gene [*24*]. We are rather inclined to think that a consideration of both environmental and genetic factors will lead to the eventual elucidation of this problem.

Dietary studies were not carried out in the course of the current investigations. The earlier observations by Wilkinson and co-workers [*25*], despite the wide scatter of their data, indicated, however, that the diets of these families closely resembled what has since come to be regarded as a "typical American diet" and there is no reason to think that their dietary habits have changed in the intervening years. Wilkinson's earlier studies [*4*] and some determinations of our own established that the disorder in these families represents "hypercholesterolemia" and not "hyperlipemia."

A close relation between coronary heart disease and the disturbance associated with elevated serum cholesterol levels is indicated by the present findings. All six subjects with xanthomatosis died at an early age. Moreover, three of the eighteen men aged forty-five years and over (17 per cent) showed evidence of manifest heart disease; each of these three men had serum cholesterol levels in excess of 300 mg. per cent. In fact, only two of the five men aged forty-five years and over who had serum cholesterol levels in this high range were free of overt coronary disease. The current data are, therefore, at variance with the conclusion arrived at by Wilkinson and associates, based on their earlier study of these families, that elevation of serum cholesterol does not reflect a predisposition to coronary disease among the so-called heterozygous carriers of the abnormal gene [*6*].

Further support for the view that coronary heart disease among these families is unduly common may be derived from a consideration of prevalence rates. Among the men aged forty-five years and over, approximately 17 per cent, as already mentioned, showed evidence of coronary heart disease, a prevalence rate considerably in excess of the limited comparable data currently available. Based on age groups and diagnostic criteria similar to those employed in this study, a prevalence rate of 4.6 per cent was found in Framingham, Massachusetts [*20*], a rate of 7.3 per cent among clothing workers of Italian extraction in New York City [*21*] and rates of 5.3 and 4.3 per cent, respectively, among civil servants in Albany [*26*] and Los Angeles [*27*]. The similarity of these rates lends credence to their representativeness. Using comparable data, a higher rate (16.4 per cent) was found among randomly chosen clothing workers of Jewish extraction, similar in age and other respects to their Italian counterparts [*21*]. While the present findings suggest that hypercholesterolemia was associated with an increased risk of coronary heart disease in these families, whether in the presence or absence of xanthomatosis, it is not implied that other factors are not of equal or, under different epidemiological situations, of greater importance than disturbances of lipid metabolism in the pathogenesis of atherosclerosis.

SUMMARY

The mode of inheritance of familial hypercholesterolemia and its relation to coronary heart disease were studied in 268 persons belonging to a single family group which was heavily tainted with this abnormal trait. Analysis of the present data and those taken from the literature provided no firm basis for accepting without reservation any of the currently proposed genetic

models of hypercholesterolemia. In particular, it was left open to question whether xanthomatosis resulted from the presence of a single gene or the concurrence of two abnormal alleles; it was suggested that the less severe degrees of hypercholesterolemia reflect not only the presence of a specific genetic abnormality but also the moderating influence of additional host and environmental factors.

The disturbance related to elevated serum cholesterol levels was shown to be associated with increased susceptibility to the development of coronary heart disease, whether xanthomatosis is present or not, a finding at variance with a report based on a study of the same families ten years ago.

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Serum Lipids, Heredity and Environment*

A Study of Adult Twins

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INTEREST in the serum lipids stems in great part from the role which lipid metabolism may play in the etiology of atherosclerotic cardiovascular disease. In the presence of serum lipid levels above normal, such as characterize idiopathic hypercholesteremia, the incidence of coronary artery disease is increased [*1*]. Of equal importance, however, is the fact that high normal or even distinctly increased serum lipid levels are not always associated with atherosclerotic vascular disease, nor is the latter invariably accompanied by radical deviations in serum lipids [*2*]. Both cardiovascular disease and serum lipid levels may similarly be associated with such factors as age, sex, heredity, a variety of disease states, and possibly nutritional status and diet [*3,4*]. The present investigation is concerned with the measured levels of serum lipids, and the possible influences of heredity upon their variations.

The use of a twin study method for investigating the contribution of heredity to variations in circulating lipid levels is suggested by the nature of this problem. Serum lipids are normal blood constituents and, as such, the products of normal metabolic and enzymatic processes characterizing the species, and consequently its heritage. The problem then is clearly not whether serum lipids are inherited, as may be the case in qualitatively normal or abnormal characteristics, but whether measured variations in serum lipid levels have a discernible hereditary component.

Since the two members of a monozygotic twin pair are genetically identical, any observed differences in their serum lipid levels must be due to factors other than heredity. By studying both members of a monozygotic twin pair simultaneously, intrapair age differences are eliminated; intrapair sex differences are of course excluded since monozygotic twins are of the same sex. The effects of disease upon measured lipid levels can be controlled by selecting twins who are in good general health and who present in their medical histories freedom from serious disease. With control of these variables, other factors which may contribute to intrapair serum lipid differences are more amenable to evaluation.

Dizygotic twins differ on the average in one-half of their genetic constitution, as do single born siblings. Intrapair differences in dizygotic twins are therefore the result of influences which are comparable to those affecting monozygotic twins, with the addition of a difference in one-half of their total heredity. Thus, by comparing the intrapair differences in the two types of twins, the effect of this difference in hereditary constitution upon variations in serum lipids is obtained.

THE STUDY SAMPLE

The twins presented in this report are part of a larger study which has been in progress for several years at the Columbia-Presbyterian Medical Center [*6*] and which will be reported in detail elsewhere [*7*]. The total sample consists of 131 Caucasian adult twin pairs drawn from a variety of sources in New York City, unselected as to sex and zygosity, ranging in age from eighteen to fifty-five years, and obtained for the purpose of establishing a population of adult twins in good general health. The subjects are repre-

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sentative, in social and economic backgrounds, of a low middle to upper middle income group in New York City. Occupations include restaurant workers, clerks and sales personnel, college students, teachers and physicians. The population is approximately equally divided between Jews and non-Jews, the latter being predominantly of Northwest European descent. The proportions of these two groups were equally distributed in the different categories employed in the analysis, and no statistically significant intrapair differences were found between Jews and non-Jews in the present study.

Serum lipid determinations were obtained for 102 twin pairs. In this group there were thirty-three female and twenty-four male monozygotic twin pairs, twenty-seven female and eight male like sex dizygotic pairs, and ten pairs of unlike sex twins. This number was then reduced to eighty-two pairs for the analysis (see later).

METHODS

The central problem in any twin study is the accurate diagnosis of zygosity. The method used in the present study was based upon proving dizygosity, first by a proved difference in a blood group factor, using: A_1, A_2, B, O; M, N, S; C, D, E, c of the Rh series; Kell, Duffy and P. All determinations were made at least in duplicate to avoid the possibility of typing errors. Other reliable characteristics, such as eye color, hair color, ear form, digital hair and dermal patterns, were used for all like sex pairs agreeing in their blood factors. These criteria, as well as the method of diagnosis, have been discussed [*6*] and will be presented in detail in a forthcoming publication [*7*].

The subjects were registered as outpatients in the Vanderbilt Clinic of the Columbia-Presbyterian Medical Center. Their health histories were obtained and their health status established on the basis of a complete laboratory and medical work-up. This included a complete physical examination by a staff physician, blood studies, urinalysis and chest x-ray films. On the basis of these examinations sixteen pairs (seven female and three male monozygotic pairs, five female dizygotic pairs, and one unlike sex pair) have been excluded.

Blood specimens were drawn at the time of examination and sent to the Nutrition Laboratory at Mount Sinai Hospital for lipid determinations. Whenever possible fasting specimens were obtained, and in every instance specimens were drawn from both members of a twin pair at the same time to assure within-pair comparability. Serum cholesterol and esterified cholesterol were determined by the Sperry-Schoenheimer method [*8*], and phospholipids by Sperry's modification of the Fiske-SubbaRow method [*9*].

RESULTS

Determinations of total serum cholesterol, cholesterol esters and phospholipid were obtained for eighty-six adult twin pairs who were found to be in good general health. It was decided to eliminate from the analysis persons whose serum cholesterol levels were beyond the 95th percentile point. The application of this criterion resulted in the exclusion of four twin pairs with idiopathic hypercholesteremia (two male monozygotic and two female monozygotic pairs) whose cholesterol values ranged from 307 to 412 mg. per cent. These values were well beyond the upper 5 per cent levels determined for a similar New York City population [*10*].

The number of individual subjects in each classification, the average ages and the average serum lipid levels, expressed in mg. per cent, are presented in Table I for the 164 adult twins in good general health having cholesterol values below the 95th percentile point. Each sex and zygosity category is subdivided into two groups, representing twin pairs living together or living apart prior to and at the time of study. Twin pairs were classified as living together if the two members of a pair were sharing the same home and taking at least two meals a day together. Those twins living apart had lived in separate homes for an average period of eight years, with a minimum of six months and a maximum of thirty-two years. The twins classified as living together tended to be younger on the average than those living apart. The age difference explains the slightly higher average lipid levels in the "apart" categories [*11,12*]. The differences in both the age and average individual lipid levels between the two categories would have importance for the present study only if the age trend were reflected in the intrapair differences for serum lipids. To test this possibility multiple regressions were calculated. In no instance was a significant departure from a zero effect obtained, indicating that no age effect upon the magnitude of the intrapair differences in our sample was apparent.

The mean intrapair differences in total serum cholesterol of the twin pairs living together were compared to those of twin pairs living apart on the basis of mean intrapair variances. (Table II.) The mean intrapair variance is $\frac{\Sigma x^2}{2n}$ where x is the difference between the two members of a

TABLE I
AVERAGE VALUES FOR AGE AND SERUM LIPIDS

Residence	N*	Age	Cholesterol (mg. %)		Phospholipids (mg. %)
			Total	Ester	
(A) Monozygotic					
♂♂ Together	28	25.04	196 ± 39	149 ± 31	244 ± 34
♂♂ Apart	10	28.20	219 ± 35	160 ± 25	267 ± 52
♀♀ Together	28	23.82	198 ± 38	148 ± 26	241 ± 34
♀♀ Apart	20	29.55	208 ± 27	154 ± 21	259 ± 28
(B) Dizygotic					
♂♂ Together	12	22.50	200 ± 34	151 ± 30	242 ± 32
♂♂ Apart	4	22.50	187 ± 73	137 ± 49	190 ± 131
♀♀ Together	18	20.56	215 ± 26	159 ± 18	255 ± 28
♀♀ Apart	26	33.23	223 ± 38	163 ± 27	268 ± 34
♂♀† Together	10	20.10	192 ± 34	141 ± 23	251 ± 42
♂♀† Apart	8	29.25	218 ± 53	160 ± 35	268 ± 50

* N = No. of twin individuals.
† Represents members of unlike sex dizygotic pairs.

TABLE II
MEAN INTRAPAIR DIFFERENCES IN TOTAL SERUM CHOLESTEROL FOR TWIN PAIRS LIVING TOGETHER AND LIVING APART

	n*	Variance	F	P
(A) Monozygotic				
♂♂ Together	14	279.536	1.22	>.25
♂♂ Apart	5	340.100		
♀♀ Together	14	205.821	2.93	<.05
♀♀ Apart	10	602.050		
(B) Dizygotic†				
♀♀ Together	9	365.611	2.38	.10
♀♀ Apart	13	868.615		
♂♀ Together	5	302.500	3.61	<.10
♂♀ Apart	4	1090.625		

* n = No. of twin pairs.
† No male comparison is made, since there were only two pairs in the "apart" category.

twin pair for their measured serum lipid levels in mg. per cent, and n is equal to the number of twin pairs. From these mean intrapair variances F ratios were calculated, and the F distribution was used to obtain the probability levels of these ratios.

It can be seen that the apart variances for total cholesterol consistently exceeded the together variances. The same was true for cholesterol esters and phospholipids. Of the female monozygotic twins, the pairs living apart had a greater mean intrapair variance, significant at the 5 per cent level; the same was found in this twin category for phospholipids. Although the ratios obtained in the together: apart comparisons are small and only of borderline significance, the consistently larger apart variances suggest the importance of environmental factors upon serum lipid levels.

The question arose as to whether or not environmental factors were similarly reflected in differences of nutritional status in the two groups. For this purpose body weight differences were taken as a simple measure of intrapair differences in nutritional status. For female monozygotic twins living together the intrapair weight variance is actually greater (51.179) than for those living apart (31.400); for dizygotic females living together (80.833) and for those living apart (86.583); in neither is the difference significant. Although in the male monozygotic twins the together weight variance (103.071) is less than the apart weight variance (130.200), this difference is not significant. For unlike sex pairs the together and apart variances are 1093.500 and 268.875, respectively, again not significant in this small number of subjects. Although the weight factor may be more important in males than in females, it can be concluded that environmental factors do not have a similar effect upon average intrapair weight and serum cholesterol differences.

In Table III monozygotic twin variances are compared to dizygotic twin variances for total serum cholesterol. In no instance was a statistically significant difference found between

TABLE III

MEAN INTRAPAIR DIFFERENCES IN TOTAL SERUM CHOLESTEROL FOR MONOZYGOTIC AND LIKE SEX DIZYGOTIC TWIN PAIRS

			n*	Variance	F	P
♂♂	Together	Monozygotic	14	279.536	2.48	<.10
		Dizygotic	6	694.250		
	†Apart	Monozygotic	5	340.100	....	.....
		Dizygotic	2	.....		
♀♀	Together	Monozygotic	14	205.821	1.78	<.25
		Dizygotic	9	365.611		
	Apart	Monozygotic	10	602.050	1.44	>.25
		Dizygotic	13	868.615		

* n = No. of twin pairs.

† No. of dizygotic males apart insufficient for comparison.

TABLE IV

MEAN INTRAPAIR AND INTERPAIR DIFFERENCES IN TOTAL SERUM CHOLESTEROL

			D.F.	Variance	F	P
(A) Monozygotic						
♂♂	Together	Intrapair	14	279.536	9.95	<.001
		Interpair	18	2780.492		
	Apart	Intrapair	5	340.100	8.18	<.025
		Interpair	18	2780.492		
♀♀	Together	Intrapair	14	205.821	9.52	<.001
		Interpair	23	1960.290		
	Apart	Intrapair	10	602.050	3.26	>.025
		Interpair	23	1960.290		
(B) Dizygotic						
♂♂	Together	Intrapair	6	694.250	2.19	>.10
		Interpair	6	1519.144		
♀♀	Together	Intrapair	9	365.611	4.10	>.01
		Interpair	21	1498.178		
	Apart	Intrapair	13	868.615	1.73	<.25
		Interpair	21	1498.178		
♂♀	Together	Intrapair	5	302.500	6.68	>.025
		Interpair	8	2022.000		
	Apart	Intrapair	4	1090.625	1.85	>.25
		Interpair	8	2022.000		

monozygotic and dizygotic twins, although the dizygotic differences are consistently larger than those of the monozygotic twins. This resembles the apart variances in Table II. The consistency in the direction of the ratios of monozygotic:dizygotic and together:apart, rather than the magnitude, implies that both genetic and environmental factors influence variations (possibly in a cumulative way) in "normal" serum lipid levels.*

For the interpretation of these data two major considerations must be taken into account. First is whether the magnitude and range of measurable variation in normal serum lipid values obtained in the present study sample is adequate for a twin analysis. The second concerns the magnitude of the intrapair genetic differences in dizygotic twins, and in environmental factors between twin pairs living together and living apart. The importance of these factors can be evaluated, and the present analysis extended, by comparing the mean *intrapair* variances to the mean *interpair* variances. (Table IV.)

The mean interpair variances have been calculated from the averages of the values for the two members of each twin pair. For this purpose the pairs living together can be combined with those living apart. No significant differences are found for the average values in the two categories, and the values obtained are for unrelated pairs, all of whom are obviously living apart. The mean variances obtained from these pair averages were multiplied by two to make them comparable to the intrapair variances, since the latter are based upon individuals and interpair

* A heritability ratio could be computed from these data to indicate the proportions of the total variances which can be accredited to heredity and environment. Because of reservations concerning this ratio in human data, this computation has not been made.

variances are based upon means of pairs of individuals.

The data in Table IV indicate that the variation in measured serum lipid values is sufficient to provide a reliable test by a twin analysis. The comparability of the intrapair:interpair variance ratios to the ratios obtained in Table II indicates that there is no unusual intrapair environmental difference affecting the present data.

The analysis in Table IV compares unrelated pairings to monozygotic and dizygotic twins as well as the full range of environmental differences with the intrapair differences for twins living together and living apart. In monozygotic twins living together, genetic identity and the greatest environmental similarity are contrasted with the statistical equivalent of unrelated people. At the other extreme are the dizygotic twins living apart. This contrasts the greatest genetic and environmental dissimilarity observed in this sample with unrelated people. Whereas the first extreme provided the largest F ratios, 9.52, 9.95, the latter shows the smallest F ratios, 1.73, 1.85. Between these extremes there is a range of variation as environmental and genetic differences change in monozygotic twins living apart and dizygotic twins living together.

Comparison of the together and apart ratios in Table IV, and of table sections A with B, demonstrates the effects of both genetic and environmental influences upon total cholesterol levels and the cumulative nature of their effects. With the relatively small range of genetic and environmental differences which occurred between the two members of a twin pair these influences could not be as clearly seen in Tables II and III.

An analysis parallel to the one presented for total serum cholesterol was carried out for esterified cholesterol and phospholipids. The results obtained were comparable for all three lipid fractions, except for slightly smaller F ratios for esterified cholesterol and phospholipids. The similar pattern in these three lipid fractions would seem to imply a uniform and fundamental influence upon the entire lipid metabolism rather than upon a single lipid fraction.

COMMENT

The present analysis demonstrates that variation in serum lipid levels may result from both genetic and environmental influences. It further indicates in an adult population in essentially good health the relative magnitude of these influences necessary for producing statistically measurable variation in these lipid levels. Significant effects were obtained when the genetic and environmental differences of unrelated individuals of a New York City middle income population were contrasted to: (1) genetically identical people with environmental differences restricted by a common family background and (2) people in the first degree of relationship sharing a common home environment. The latter observation is comparable to the findings of a study of families reported by Adlersberg, Schaefer and Steinberg [*13*]. One may deduce from these observations that the environmental tolerances of one population will not equal those of another population, and that genetic factors will not be equally important in all environments.

There is a suggestion that the genetic and environmental factors influencing serum lipid levels in twins living together and living apart, and in monozygotic and dizygotic twins, are essentially similar and differ only in degree. The socioeconomic differences are on the average greater between pairs than between two members of any given pair. Despite the difference between pairs, every person in the sample was able to provide himself with an adequate diet, the fat, protein and carbohydrate content of which was dictated only by taste and needs. In a recent study of a New York City population Epstein et al. [*14*] found that Jewish and Italian employees in the clothing industry had very similar caloric and total fat intakes in spite of differences in dietary habits. One wonders whether or not dietary differences constituted the largest environmental variable. Certainly for twins living together and living apart one may assume that in this twin sample the intrapair differences in diet must be quite similar, and yet for those living apart consistently larger average lipid variances were seen. Twin pairs living apart show greater occupational differences than those living together. For example, in the monozygotic pair with the second largest difference in total serum cholesterol, one member was a fireman and the other member a steamship agent.* A difference in occupation such as this may be reflected in environ-

* The fireman's total serum cholesterol was 249 mg. per cent and that of the steamship agent was 218 mg. per cent.

mental attributes other than in food intake alone.

CONCLUSIONS

A twin method has been employed for the study of the role of genetic and environmental factors in the regulation of serum lipid levels. By this method it has been possible to demonstrate that both genetic and environmental factors may produce measurable variations in the serum lipid levels of healthy adults. Although the manner of the genetic and environmental interaction cannot be determined from the present data, it appears unlikely that diet constitutes the only environmental factor affecting serum lipid levels.

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The Mode of Inheritance in Essential Familial Hypercholesterolemia*

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SINCE the first description of xanthelasma by Rayer in 1836 [*1*] and the first reported case of xanthomatosis by Addison and Gull in 1851 [*2*], several hundred cases of presumably primary xanthomatosis have appeared in the literature. The first recognition of the familial incidence of xanthomatosis was by Startin in 1882 and by Mackenzie in the same year [*3a,3b*]. The first recognition of the relationship of serum cholesterol to this disease was by Pick and Pinkus in 1908 [*4*], although the first clinical studies of serum cholesterol in such patients did not come until the case reports of Schmidt in 1922 [*5*].

The great importance of differentiating xanthomatosis caused by primary elevation of serum cholesterol without elevation of neutral fat, from that caused by hyperlipemia, in which neutral fat elevation is the primary abnormality found in the serum, was first stressed by Thannhauser and Magendantz in 1938 [*6*] and by Thannhauser in 1940 [*7*]. The concept that moderate elevation of serum cholesterol in the relatives of those patients with xanthomatosis represents a heterozygous or "carrier" state, although suspected by several other investigators earlier, received a great deal of factual support by Wilkinson, Hand and Fliegelman in 1948 [*8a,8b*] in their report on the largest kindred to be found in the literature to date. We shall follow their terminology and call this disease essential familial hypercholesterolemia (E.F.H.).

A great number of excellent reviews of the subject are available, the most extensive of which are listed in the bibliography [*9–18*†]. On examination of these reviews and of case reports, one discovers two leading schools of thought regarding the genetics of E.F.H. One group of investigators, represented in the last few years mainly by Piper and Orrild [*19*], Alvord [*20*], Leonard [*21*] and Harris-Jones et al. [*22*], and formerly by Mueller [*23*], Kornerup [*24*] and Stecher and Hersh [*25*], maintains that inheritance of the trait can be explained on the basis of a simple dominant gene and that xanthomatosis and hypercholesterolemia are but different expressions of the same gene. The other group, represented principally by Wilkinson et al. [*8a,8b*], Herndon [*18*], Neel and Schull [*26*], Adlersberg et al. [*27*] and Godal et al. [*28*], is of the opinion that E.F.H. is inherited by transmission of an incompletely dominant gene. A few authors, such as Levin and Sullivan [*29*], believe the mode of transmission to be that of a recessive gene, but this concept is no longer considered feasible in the light of modern evidence.

Incomplete dominance and incomplete recessiveness mean the same thing, and refer only to whether the gene was first thought to be dominant or recessive [*26*]. For this reason, the proper terminology in this instance is probably "incompletely dominant." This gene produces moderate elevation of serum cholesterol, when present in a single dose (heterozygous), and marked elevation of serum cholesterol, severe xanthomatosis, and increased susceptibility to coronary heart disease and early death by myocardial infarction when present in a double dose (homozygous).

We shall report in this paper the findings in one large and two smaller kindreds (Figs. 1, 2 and 3 and Tables I, II and III) which support the hypothesis that E.F.H. is transmitted by an incompletely dominant gene.

METHOD

A complete medical history was obtained and a complete physical examination performed on all sub-

† The only report of monozygotic twins with E.F.H. in the literature is that of Bruins [*30*].

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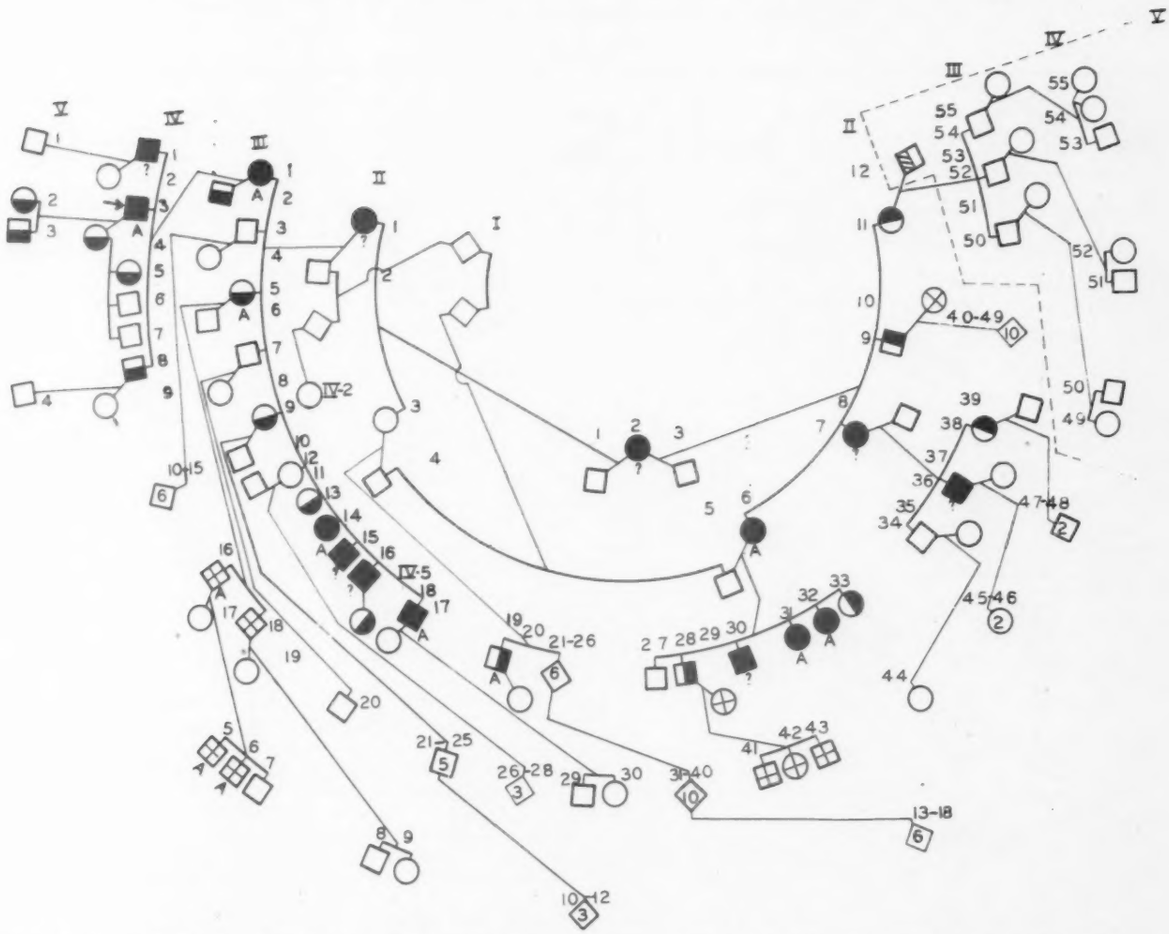


FIG. 1. Pedigree 1. The broken line shows the marriage of a heterozygous hypercholesterolemic person and a heterozygous hyperlipemic person. This group is to be reported separately. For further explanation see "Addendum."

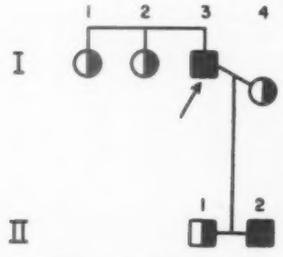
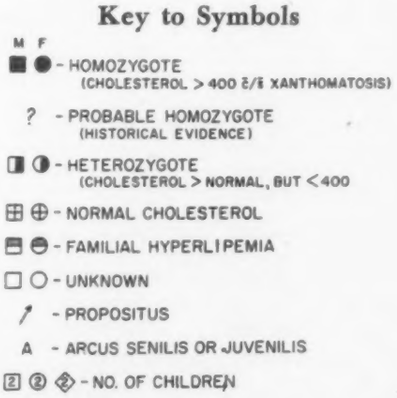


FIG. 2. Pedigree 2.


Key to Symbols
M F
- HOMOZYGOTE (CHOLESTEROL > 400 ± XANTHOMATOSIS)
? - PROBABLE HOMOZYGOTE (HISTORICAL EVIDENCE)
- HETEROZYGOTE (CHOLESTEROL > NORMAL, BUT < 400
- NORMAL CHOLESTEROL
- FAMILIAL HYPERLIPEMIA
- UNKNOWN
- PROPOSITUS
A - ARCUS SENILIS OR JUVENILIS
- NO. OF CHILDREN

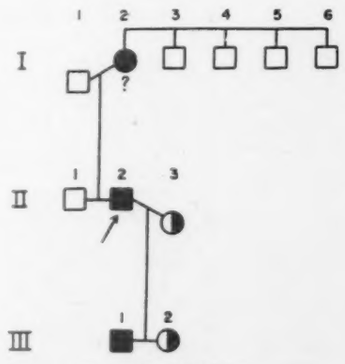


FIG. 3. Pedigree 3.

jects. Blood samples were drawn in the fasting state, permitted to clot, centrifuged, and the serum kept at refrigerated temperature until the time of the chemical determinations. This interval was never longer than ten days after the drawing of the samples, and in most cases was less than one week. More than half of the subjects had two or more separate samples drawn on different occasions. In pedigree 1 the propositus (IV, 3) and two others of the most severe cases (III, 14 and 17), as well as subjects I, 3 and II, 2 of pedigree 2 and II, 2 of pedigree 3, were hospitalized, and the following studies were made: complete blood count, complete urine analysis, blood sugar (fasting and two hours postprandial), protein-bound iodine,

TABLE I
PEDIGREE 1

No.	Age (yr.) Now or at Death	Sex	Total Cholesterol (mg. %)	α to Total Lipoprotein	Blood Type and Rh	Comments
			Group I			
1.	?*	M	....	..	....	Said to have died young
2.	80*	F	....	..	....	Said to have had xanthomas
3.	62*	M	....	..	....	Said to have died of heart attack
			Group II			
1.	78*	F	....	..	....	Said to have had large xanthelasma
2.	79*	M	....	..	....	.....
3.	62*	F	....	..	....	Said to have died of heart attack
4.	73*	M	....	..	....	.....
5.	57*	M	....	..	....	Said to have died of heart attack
6.	68	F	796	16	A+	Xanthelasma, xanthoma tendinosum of hands, angina ten years, gallbladder disease, aortic systolic murmur
7.	49*	F	....	..	....	Said to have had large xanthelasma and to have died of heart attack
9.	74	M	278	24	....	.....
11.	71	F	347	20	0+	Tiny xanthelasma, gallbladder disease
12.	75	M	....	..	....	See note at III, 50–55, hyperlipemia
			Group III			
1.	65	F	644	19	AB+	Xanthoma tendinosum of hands, xanthoma tuberosum of bridge of nose, arcus senilis, hypertension 6 years (200/110 mm. Hg), aortic systolic murmur radiating to neck
2.	68	M	326	21	O+	Gallbladder disease
5.	59	F	273†	26	A+	Physical and history negative
6.	69	M	309	24	O+	Had hypothyroid heart disease until given thyroid gr. 1½ O.D. 7 years ago, thyroid status unknown
9.	57	F	330	27	AB+	Psoriasis
13.	50	F	312	37	A+	Tiny xanthelasma of one eye
14.	49	F	538	20	AB+	Xanthoma tendinosum of hands, knees, heels, xanthoma tuberosum of bridge of nose, arcus senilis
15.	47	M	....	..	....	Said to have xanthomas
16.	37*	M	....	..	....	Died of myocardial infarction, had xanthomatosis.
17.	38	M	536	10	AB+	Xanthoma tendinosum of hands, xanthoma tuberosum of elbows, arcus juvenilis, systolic apical murmur, recent attacks of weakness, electrocardiographic evidence of myocardial damage.

TABLE 1 (*Continued*)
PEDIGREE 1

No.	Age (yr.) Now or at Death	Sex	Total Cholesterol (mg. %)	α to Total Lipoprotein	Blood Type and Rh	Comments
19.	54	M	266†	27	A+	Arcus senilis
27.	23*	M	....	..	....	Died of tuberculosis
28.	46	M	267	19	AB+	Tiny xanthelasma of one eye
29.	40	F	218	30	A−	Physical and history negative
30.	34*	M	....	..	....	Died of myocardial infarction
31.	40	F	468	15	A+	Arcus juvenilis, aortic systolic murmur
32.	35	F	548	33	A−	Arcus juvenilis
33.	28	F	271	31	B+	Physical and history negative
36.	39*	M	....	..	....	Said to have had large xanthelasma and to have died of heart attack
50. to 55.	...	..	....	..	....	Descended from 1 hypercholesterolemic and 1 hyperlipemic parent, group to be carefully studied and reported in the future
			Group IV			
1.	38*	M	....	..	....	Died of myocardial infarct, had xanthomatosis
3.	45	M	570	13	B+	Propositus, xanthoma tendinosum of hands, knees, heels, xanthoma tuberosum of elbows, toes, bridge of nose, multiple sclerosis-like syndrome of periodic weakness and scotomas
4.	43	F	284	35	A+	Physical and history negative
5.	45	F	240†	25	A+	Physical and history negative
6.	47	M	....	15	....	Multiple subcutaneous lipomas on face, chest and back
7.	48	M	....	..	....	Recent myocardial infarct
8.	39	M	333†	20	A+	.....
16.	35	M	209†	25	A+	Arcus senilis
18.	33	M	223	17	A+	Physical and history negative
41.	14	M	187	25	B+	Physical and history negative
42.	13	F	177	34	AB−	Physical and history negative
43.	12	M	250	29	B+	Physical and history negative
49 to 55.	...	..	....	..	....	See comment under III, 50–55
			Group V			
2.	15	F	276†	19	O+	Physical and history negative
3.	13	M	337†	38	B+	Systolic apical murmur
5.	12	M	195	37	O+	Arcus juvenilis
6.	11	M	176†	29	O+	Arcus juvenilis

* = Age at death.
† = Sperry method.

TABLE II
PEDIGREE 2

No.	Age (yr.) Now or at Death	Sex	Total Cholesterol (mg. %)	α to Total Lipoprotein	Blood Type and Rh	Comments
			Group I			
1.	50	F	345*	..	..	Cholesterol determination performed at another laboratory, said to have no xanthomas
2.	48	F	370*	..	..	Cholesterol determination performed at another laboratory, said to have xanthelasma
3.	44	M	620†	..	..	Propositus, xanthoma tendinosum of hands, heels, xanthoma tuberosum of buttocks, elbows, xanthelasma, angina pectoris for 20 years, electrocardiographic evidence of myocardial damage
4.	45	F	280†	..	..	Physical and history negative
			Group II			
1.	14	M	294†	..	..	Physical and history negative
2.	11	M	702†	..	..	Xanthoma tuberosum of one elbow

* = Bloor method.
† = Sperry method.

urine urobilinogen excretion, fat tolerance tests to definitely rule out hyperlipemia, electrocardiogram and chest x-ray.

The following determinations were performed on the fasting specimens drawn from all subjects of pedigrees 1 through 3: (1) Serum cholesterol: Method in the majority of cases: Zak et al. [*31*]. Upper limit of normal: 260 mg. per cent. In some cases the Sperry-Webb modification of the method of Schoenheimer and Sperry [*32*] was used. Upper limit of normal: 210 mg. per cent. (2) Phospholipid as lipid phosphorus by the method of Simonsen et al. [*33*]. (3) Total fatty acids by the method of Bauer and Hirsch [*34*]. (4) Alpha and beta-lipoproteins by the method of Jencks and Durrum [*35*]. Lower limit of normal for alpha-lipoproteins is 20 per cent of total. (5) ABO and Rh blood grouping to aid in determining paternity.

RESULTS

The results are summarized in Tables I through III. The reference numbers, showing the relationship of the subjects to each other refer to the pedigrees. (Figs. 1, 2 and 3.) The results of the aforementioned special investigations on the hospitalized subjects were all negative with the exception of evidence of myocardial damage in the electrocardiograms of subject III, 18 of pedigree 1, although he gave no history of any apparent cardiac episode, and of subject I, 3 of pedigree 2, who had a twenty-year history of angina pectoris.

COMMENTS

On examination of all three pedigrees it becomes apparent that no person whom we have labelled homozygote has produced any normal offspring, that all homozygotes have two abnormal parents when those parents were available for examination, and that the ratios between homozygotes, heterozygotes and normal subjects among the offspring of the various marriages correspond to the ratios expected from inheritance by an incompletely dominant gene [*8a*]. We believe this theory explains adequately the inheritance in these three kindreds, as well as in the kindreds reported in the literature.

So far as the concept of complete dominance in this condition is concerned, it is of course possible that there are two forms of inheritance which produce a similar or even completely identical disease, that is, identical within the limits of our clinical diagnostic ability. This may

TABLE III
PEDIGREE 3

No.	Age (yr.) Now or at Death	Sex	Total Cholesterol (mg. %)	α to Total Lipoprotein	Blood Type and Rh	Comments
			Group I			
1.	52*	M	....	..	..	Carcinoma of lung
2.	63*	F	....	..	..	Had xanthomatosis, died of myocardial infarct
3.	76*	M	....	..	..	Cerebrovascular accident
4.	55*	M	....	..	..	Carcinoma of rectum
5.	49*	M	....	..	..	Myocardial infarct
6.	39*	M	....	..	..	Brain tumor
			Group II			
1.	57*	M	....	..	..	Myocardial infarct
2.	53	M	420†	..	..	Propositus, xanthoma tendinosum of hands, knees, xanthoma tuberosum of elbows, angina 3 years
3.	50	F	376	..	..	Physical and history said to be negative
			Group III			
1.	27	M	490	..	..	Physical and history negative
2.	23	F	278†	..	..	Soft apical systolic murmur

* = Age at death.
† = Sperry method.

occur in the form of two completely different and unrelated genes, or in different mutations at the same locus. It is, however, important to remember that in all the literature on medical genetics there is no single clear-cut report of a homozygote for any of the rare so-called completely dominant genes. Even these diseases may be inherited by incomplete dominance in that the homozygote may manifest a much more severe form of the disease, even to the point of being lethal [*27*].

Several criticisms may be leveled at some of the kindreds reported to show "complete" dominance. Perhaps the most pertinent of these is the use of an upper limit of normal serum cholesterol which has since been shown to be too high, especially by the excellent study of Linholm [*36*]. Although this study applies to the Sperry-Schoenheimer method for cholesterol, the well known interrelationship between the values obtained by this method and by others used by various authors leads one to the conclusion that, in general, the upper limits of normal were taken as too high. In several of the reports, use of a more acceptable upper limit of normal leads to the conclusion that the mode of inheritance appears to correspond to incomplete rather than to complete dominance. Piper and Orrild [*19*] did not examine their new patients for hyperlipemia, and adopted what is probably too high a normal limit for cholesterol. Alvord [*20*] is dealing with a kindred in which hypercholesterolemia and hyperlipemia are both present in various individuals, and uses too high an upper limit of normal for cholesterol. Leonard [*21*] introduces a bias into his kindred by calling

one group of cholesterol values normal when xanthomatosis is not present and abnormal when xanthomatosis is present, although the cholesterol level in both is elevated. Harris-Jones [*22*] uses too high an upper limit for normal for cholesterol and, unfortunately, has no paternity checks on the one subject who may prove the theory of "complete" dominance. It is also important to emphasize again, that not only is xanthomatosis alone found in homozygous E.F.H., but also a markedly elevated cholesterol with severe xanthomatosis and, frequently, early death from coronary disease.

We fully realize that in the younger subjects of these kindreds xanthomatosis may not yet have appeared but they have a much more marked elevation of serum cholesterol than their heterozygous relatives and, if they live long enough, xanthomatosis will almost certainly develop.

We have included studies on the ratio of serum alpha- to beta-lipoproteins. This ratio, together with the fact that the females in these kindreds may live to a relatively old age, seems to indicate that women are perhaps protected to some degree by their particular endocrine make-up from the ill effects of this disease to a greater extent than men. Even those women who are severely affected have, on the whole, a relatively higher percentage of alpha-lipoprotein, a theoretically "good" relationship of lipoproteins, when compared to the men who are severely affected to a like degree.

That point in pedigree 1 in which familial hyperlipemia is introduced by II, 12, and apparently inherited by his children and grandchildren is not included in this paper and will be reported in the future, when a more detailed study will have been completed. There was no hyperlipemia in any other member of any of the three kindreds.

SUMMARY

1. Some of the recent literature on essential familial hypercholesterolemia is reviewed.

2. Three additional kindreds with this disease are reported.

3. The debate relating to the genetics of this disease is reviewed, and it is concluded that the majority if not all the kindreds so far reported show inheritance of the trait through an incompletely dominant gene. The possibility that there are two forms of inheritance in this disease is considered.

ADDENDUM

Since this paper was submitted for publication additional information has been obtained. II-4 and II-5 are brothers and are first cousins of II-2. IV-2 is a niece of II-2.

This information and other probable intermarriages may explain the high gene frequency in this kindred.

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The Effect of Lipemia upon Coronary and Peripheral Arterial Circulation in Patients with Essential Hyperlipemia*

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In some patients with atherosclerosis and angina pectoris, postprandial hyperlipemia can precipitate an anginal attack [*1–5*]. The present study was undertaken in a group of six patients with essential hyperlipemia, a metabolic disease in which the fasting serum triglyceride level is elevated markedly, and which is known to be associated with the premature development of atherosclerosis [*5–9*] and with episodes suggesting transient ischemia in the myocardium, extremities, brain and abdomen [*5,6,8,10–16*]. It was designed with a twofold purpose: (1) to induce changes in the serum lipids, especially the triglyceride levels, by varying the fat intake, and (2) to correlate the changing serum lipid levels with the subjective and objective evidences of cardiac and peripheral ischemia.

MATERIALS AND METHODS OF STUDY

Six patients with essential hyperlipemia were studied, four males and two females. The four males ranged from thirty-three to sixty-two years of age and the two females were thirty-four and thirty-five, respectively. Over various periods of time four of these patients were admitted to the Metabolic Ward of the Hospital of the University of Pennsylvania for dietary control and blood lipid studies; the other two, whose symptoms of arterial insufficiency were milder, were similarly studied on an ambulatory basis. Not included in this study were three more patients, two because they had no clinical evidence of arterial disease, and one because he had a slow response to changes in dietary fat intake.

Control. The control period was from three to eight months during which all six patients consumed full diets. During this time several fasting and five- to six-hour postprandial blood samples were withdrawn for cholesterol, phospholipids and total esterified fatty acid determinations. The methods of Abell, Levy, Brodie and Kendall [*17*], Zilversmit and Davis [*18*], and Stern and Shapiro [*19*] were used, respectively. From these lipid studies the serum triglyceride was calculated. Plasma turbidity was determined by means of a Coleman junior spectrophotometer. In conjunction with the serum lipid determinations, the cardiovascular status of the patients was determined periodically by (1) ballistocardiographic studies with a Starr-type ballistocardiograph, (2) the electrocardiographic response to standard exercise (Master's two-step test), (3) the "claudication time" test according to the criteria of Barker, Brown and Roth [*20*], and (4) changes in clinical symptoms.

Experiment. At the conclusion of the control period the patients were given a modified "fat-feee" diet, as described by Mellinkoff, Machella and Reinhold [*29*], for three to twelve weeks. During this "de-fatting" period the diet consisted of a protein hydrolysate mixture, rice, fruits and vegetables, with an estimated total fat intake of less than 5 gm. per day. The patients were encouraged to consume enough calories to keep their weight fairly constant, with daily weighing. Following the control of lipemia, each patient was returned briefly to a period of high fat feeding, (one to three weeks) on either a full or a high fat diet. (The full diet contains 100 to 120 gm. of fat, the high fat diet 200 to 220 gm. of fat per day.) A second "de-fatting" period was then instituted. Finally, the patients were maintained on a diet containing 5 to 20 gm. of fat per day for three to forty-five months. The serum lipid levels were correlated with the clinical status of the patients throughout all the periods of dietary manipulation.

RESULTS

Clinical Data. The pertinent clinical data on the six patients with essential hyperlipemia and

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TABLE I

SUMMARY OF CLINICAL DATA ON SIX HYPERLIPEMIC PATIENTS WITH CLINICAL ATHEROSCLEROSIS

Patient	Sex	Age When Diagnosed (yr.)	Present Age (yr.)	Coronary Artery Disease	Peripheral Vascular Disease	Abdominal Crisis	Neurological Changes
G. H.	M	32	36	+	−	−	−
R. J.	F	30	34	+	−	−	−
M. R.	M	49	52	+	+	−	Transient
J. H.	M	31	33	−	+	Mild	−
J. C.	F	33	35	+	−	−	−
M. M.	M	61	62	+	−	−	−

clinical atherosclerosis are summarized in Table I. Five of the six patients had evidence of coronary artery disease and mild to severe degrees of angina pectoris; one of these also had intermittent claudication. One patient had intermittent claudication only. An episode of transient neurological changes suggestive of localized cerebral ischemia was observed in one patient, and two mild attacks of abdominal crisis were experienced by one other patient, while they were on a high daily fat intake. The serum lipid values obtained at the end of each experimental period are presented in Table II. It is apparent that fairly large changes in the serum lipid levels, particularly the triglyceride levels, could be induced in each patient over a relatively short period of time by the use of (1) "fat-free" (F.F.), (2) full (F.), (3) high fat (H.F.) diets. Study of the relations between the changing blood lipid values and the clinical findings indicates: (1) In a given patient, subjective and objective findings of arterial insufficiency were found to vary with the rapid rise and fall of the plasma lactescence and the serum triglyceride levels during the experimental periods. If the reduction of lipemia was maintained, progressive improvement of arterial insufficiency of the patient occurred. (2) We were unable to establish a critical serum lipid level at which all of the six patients with hyperlipemia would develop clinical signs and symptoms of arterial insufficiency. (3) The arterial oxygen saturations of two patients with coronary disease, angina pectoris and mild congestive failure were 92.1 per cent and 92.6 per cent, respectively, at the height of lipemia. Following treatment, and clearing of the lipemia, the arterial oxygen saturation of these two patients rose to 95.8 per cent and 96.1 per cent, respectively.

TABLE II

SERUM LIPID VALUES OF SIX PATIENTS AT THE END OF EACH DIETARY EXPERIMENTAL PERIOD

Patient	Date	Serum Total Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	Triglyceride (mg./100 ml.)	Diet*
G. H.	1/8/54	313	328	881	F
	1/28/54	276	270	193	FF
	2/18/54	311	305	833	F
	5/11/54	222	265	161	FF
R. J.	2/10/54	403	340	730	F
	3/1/54	257	323	223	FF
	3/20/54	368	320	596	F
	4/2/54	264	325	228	FF
M. R.	2/10/55	519	518	1,484	F
	3/4/55	273	482	389	FF
	3/27/55	472	380	891	F
	4/18/55	270	402	104	FF
J. H.	7/1/56	352	346	925	F
	7/20/56	248	240	227	FF
	7/27/56	320	312	785	HF
	8/20/56	280	290	258	FF
J. C.	8/3/56	570	550	1,466	F
	8/25/56	310	368	205	FF
	9/4/56	402	550	708	HF
	9/25/56	262	405	88	FF
M. M.	11/20/57	750	748	1,995	F
	12/11/57	439	323	363	FF
	12/21/57	524	535	1,180	HF
	1/13/58	257	308	374	FF

* F = full diet.
FF = fat-free diet.
HF = high-fat diet.

Electrocardiographic Response to Exercise. Four of the five patients with coronary artery disease and angina pectoris had normal electrocardiograms taken when they were at rest. Patient R. J. had minor depressions of T waves in leads I, II and lateral chest leads. A positive exercise test was obtained in all five patients while they were on a full diet when their serum triglyceride levels were high. With the clearing of lipemia by fat restriction all these five patients promptly experienced subjective improvement in exercise tolerance and decrease in the frequency of angina pectoris; objectively, the previously positive electrocardiographic response to exercise test became less abnormal in three to four

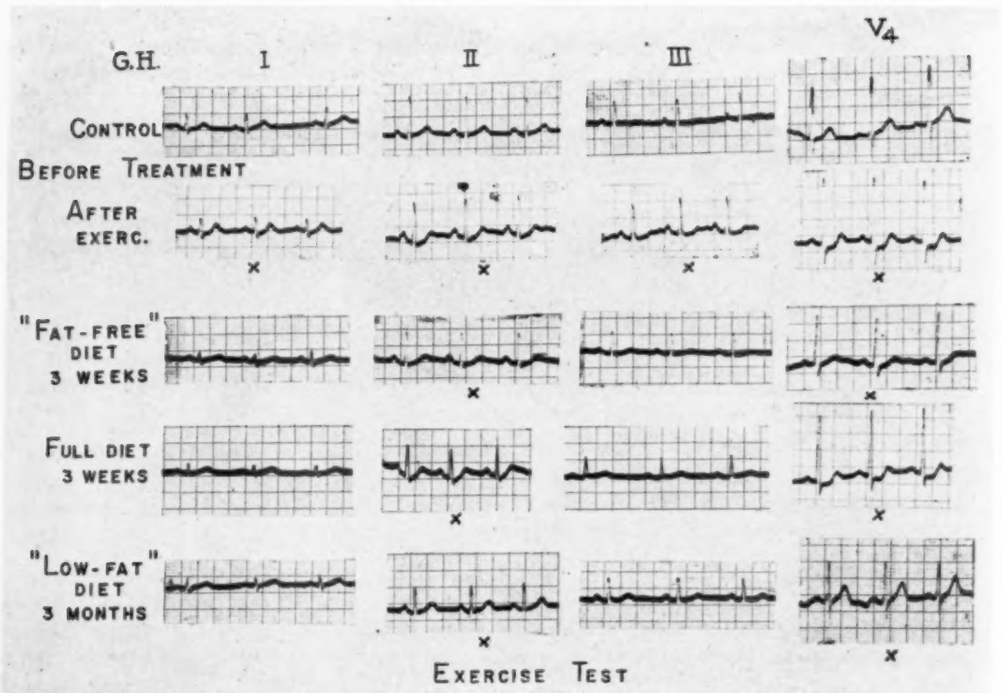

FIG. 1. Serial electrocardiographic exercise tests performed on patient G. H. during control, "fat-free," "high fat" and "fat-free" dietary control periods. X indicates tracing obtained after exercise.

weeks. If the low serum triglyceride levels were maintained, continued improvement in both the subjective and objective findings of coronary arterial insufficiency was observed. In Figure 1 are given graphic studies of one patient (G. H.), a thirty-six year old man with hyperlipemia, coronary artery disease and angina pectoris. On initial admission to the clinic in February 1954, his fasting serum triglyceride was 881 mg. per cent (normal value 85 to 350 mg. per cent), and his serum total cholesterol and phospholipids were 313 and 328 mg. per cent, respectively. On a "fat-free" diet his serum lipids values were lowered to within normal limits in three weeks. When he was briefly returned to a full diet his serum lipids, particularly the triglyceride fractions, rose rapidly to abnormal levels (833 mg. per cent). The lipemia was again brought under control by strict dietary fat restrictions. His serial electrocardiograms are also shown in Figure 1. During the initial hyperlipemic period an electrocardiogram, taken while he was at rest, was normal. He complained of substernal pressure at the end of the standard exercise test. RS-T segment depressions were observed in leads II and V_4 electrocardiograms taken immediately after the exercise. At the end of the first "de-fatting" period a similar exercise test failed to precipitate angina pectoris in this patient and the leads II and V_4 electrocardiograms taken after exercise merely showed some lowering of the T waves. Tightness in the chest and RS-T segment depressions in the electrocardiogram following exercise occurred again with the return of hyperlipemia. Reinstitution of "fat-free" diet quickly controlled the lipemia. This restriction was maintained for three months. Another electrocardiographic exercise test performed at the end of the three-month period was negative.

Response to Standard "Claudication Time" Test. Two hyperlipemic patients had intermittent claudication in the calf muscles when walking. Figure 2 gives the correlation between the serum lipid levels and the walking tolerance of patient J. H. In the eight-month control period his serum triglyceride ranged between 690 to 925 mg. per cent, together with moderately elevated serum total cholesterol and phospholipids values (352 and 346 mg. per cent, respectively). While his serum triglyceride level was markedly elevated his standard claudication time was found to range between ninety to ninety-five seconds. On clearing of the lipemia by fat restriction his exercise tolerance increased in thirteen days from ninety to ninety-five sec-

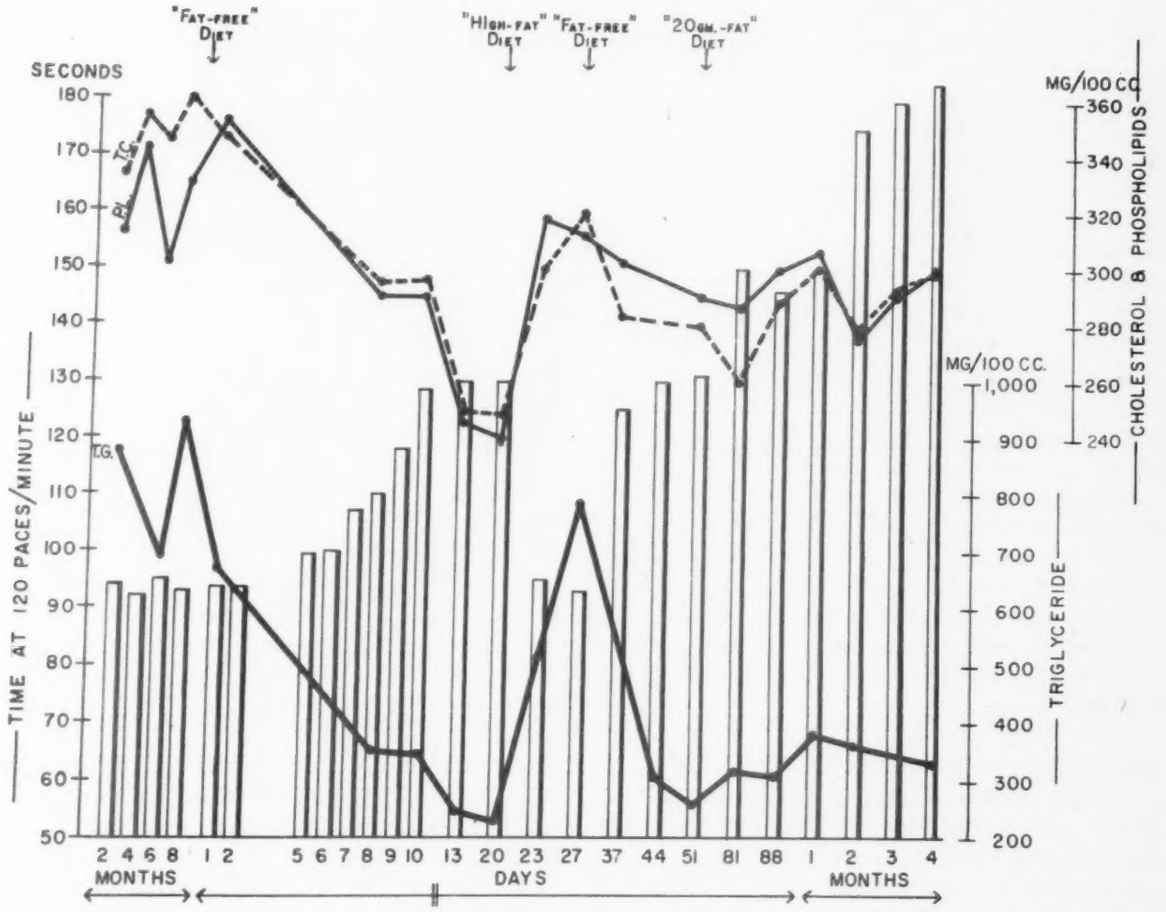

FIG. 2. Correlation of changes in serum lipid levels with dietary manipulations and with the "claudication time" of patient J. H. T.C. = total cholesterol; P.L. = phospholipids; T.G. = triglyceredes; bars repeat claudication time.

onds to 130 seconds. His walking tolerance was maintained at this level for one week while he was maintained on the "fat-free" diet. A high fat diet for a week resulted in rapid increase in his blood lipids, with a concomitant decrease in the claudication time. Following these brief experimental periods his lipemia was controlled on a long-term basis by a diet containing 5 to 10 gm. of fat per day. Once again his claudication time rapidly rose to a plateau of about 130 seconds soon after the lipemia was cleared. This early improvement was followed by a progressive secondary increase in the walking tolerance to 180 seconds during the following four months of observation. Although the claudication time of a given patient does not remain at a permanent level, the rapid changes observed with multiple determinations made on this patient must be regarded as significant.

Ballistocardiographic Changes. Ballistocardiograms with lead II electrocardiogram and pneumogram were taken in all six patients before and at the end of each dietary control period, and also at three- to six-month intervals throughout the prolonged follow-up period. The five hyperlipemic patients with coronary artery disease and angina pectoris had very abnormal ballistocardiograms prior to the use of "fat-free" and low fat diets, while their serum triglyceride concentrations were high. Figure 3 is an example of a ballistocardiogram made on patient J. C., a thirty-five year old hyperlipemic woman with coronary disease and frequent attacks of angina pectoris. While on a full diet her fasting serum triglyceride was elevated to 1,466 mg. per cent. Her serum cholesterol and phospholipids also were quite high. (Table II.) All the waves in her ballistocardiogram, especially the I and J waves, were low in amplitude. (Figure 3, top.) Except for the prominent K waves, other ballistic

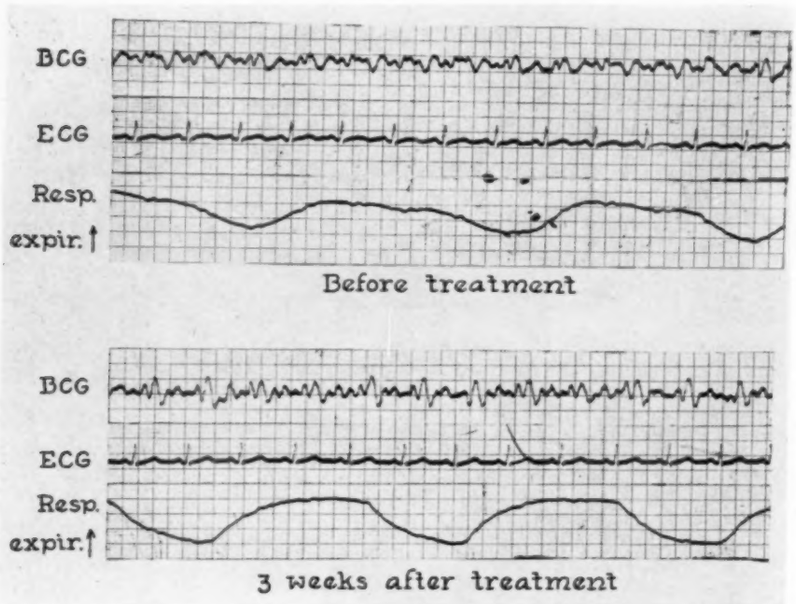

FIG. 3. Top, ballistocardiogram, pneumogram and lead II electrocardiogram taken simultaneously on patient J. C. before institution of "de-fatting" diet. Bottom, similar study made on patient J. C. three weeks after the start of "fat-free" diet.

movements are not well recorded during the relatively long and slightly jerky expiratory phase of the respiration. Three weeks after the start of a "fat-free" diet and with the clearing of lipemia (serum triglyceride 205 mg. per cent) a significant improvement was noted in her ballistocardiogram. (Fig. 3, bottom.) The tracing shows: (1) an increase in the distinctiveness and in the amplitudes of all the ballistic waves; (2) minimal variation of the ballistic complex with respiration; and (3) more regular and smoother respiratory excursions than in Figure 3, top.

It has been of considerable interest to us that following the initial phase of change little further improvement was observed in the subsequent serial ballistocardiograms, in some for as long as forty-five months.

Neurological Changes and Abdominal Crisis. Two patients, one of whom had an episode of cerebral ischemia and two attacks of abdominal pain during the initial periods of observation, did not experience further cerebral and abdominal symptoms after they were put on a low fat diet for eighteen and for forty-five months, respectively.

The Effects of Heparin Administration and Fat Ingestion. Intravenous injection of 5 to 10 mg. of heparin caused a decrease of 21 to 167 mg. per cent in the serum triglyceride level in four of the six patients. In the other two, a slight elevation of the serum triglyceride level occurred, amounting to 34 to 65 mg. per cent. It is apparent that the drug was relatively ineffective in clearing the lipemia of these patients. Furthermore, these relatively minor changes induced by heparin did not produce a significant improvement of the ballistocardiogram or of the electrocardiographic exercise response; moreover, the claudication time was unchanged during the thirty-minute to two-hour period immediately after the intravenous injection of heparin. Four to five hours after the ingestion of a standard fatty meal all of these patients showed an increase in lipemia amounting to 130 to 335 mg. per cent increase in serum triglyceride. These increments in the serum triglyceride level did not significantly increase the abnormalities in the ballistocardiogram, electrocardiogram and exercise tolerance test of these patients. Subjectively, two patients with coronary disease and angina pectoris reported that their exercise tolerance was decreased during the postprandial period.

POSSIBLE MECHANISM BY WHICH HYPERLIPEMIA CAN PRODUCE THE PHENOMENA DESCRIBED

In our early work we believed that hyperlipemia can interfere in some way with the diffusion

of oxygen from coronary vessels to the myocardium [*1,2*]. Since then we have observed an additional pulmonary factor, as indicated by the following facts: (1) both dogs and patients with hyperlipemia have a peculiar and different respiratory excursion [*1,2,23*], and (2) a slight degree of arterial oxygen unsaturation has been observed in two of our hyperlipemic patients. Consequently, we wonder whether or not hyperlipemia might act on one hand to interfere with the diffusion of oxygen into the blood from the lungs, and on the other to interfere with the diffusion of oxygen from the capillary blood to the myocardium and skeletal muscles.

COMMENTS

Signs and symptoms suggestive of episodes of acute cerebral, cardiac and visceral arterial insufficiencies have been observed in a high percentage of the reported cases of essential hyperlipemia [*6–8,10–14*]. Although Martt [*5*], Holt [*10*], Klatskin [*11*], Wang [*13*] and Adlersberg [*21*], and their associates, have suggested that these clinical manifestations may be closely related to the high neutral blood fat content of these patients, other investigators [*14,22*] doubted that a causal relationship actually exists between lipemia and the acute symptoms of arterial insufficiency observed because: (1) episodes of arterial insufficiency were encountered in only a certain number, not in all patients with hyperlipemia; (2) it had not been possible to establish a critical lipemia level that would precipitate an attack of arterial insufficiency; and (3) the onset of clinical symptoms could not be strictly correlated with the plasma triglyceride content. The present study indicates that while a definitive change in the circulatory status was not demonstrable in those patients with relatively minor changes in their blood lipids following a single test meal or after an intravenous injection of heparin, measurable changes in their clinical condition were regularly present after the induction of large changes in the serum triglyceride and particularly of the plasma lactescent levels of these patients.

The question still remains whether serum cholesterol and phospholipid levels, rather than the triglyceride content, may account for the changes observed in these patients. A partial answer may be given by the fact that in another group of four hypercholesteremic and phospholipemic patients whom we subjected to similar studies over a period of three to six weeks, little or no change could be detected in their clinical picture. The possibility has not, however, been excluded that the high carbohydrate content in the "fat-free" diet might in some way have exerted a favorable influence upon the circulation of these patients.

Going along with the general belief that changes in the ballistocardiogram reflect primarily changes in the myocardial contraction, we initially tended to ascribe the ballistocardiographic abnormalities of the hyperlipemic patients to the effect of chylomicrons upon the myocardium. However, both our clinical and laboratory studies showed ballistic movements that could not be so ascribed. For example, in patients with postprandial angina pectoris we had noted the prompt disappearance of a large number of extraneous waves when the ballistocardiograms were made while the patients were holding their breath [*1*]. Later, in the laboratory, we observed that after experimental coronary artery ligation in dogs with myocardial infarction, intravenous fat infusion caused hyperventilation and lowering of arterial oxygen saturation [*23*]. In this present study most of the ballistocardiographic abnormalities were recorded in patients during their lipemic periods while their respiratory excursions were exaggerated and jerky. Coincidental with the return of their respiration movements to a more normal level after the clearing of their lipemia, the majority of the abnormalities in the ballistocardiogram completely disappeared. Hence, when the ballistocardiogram was made with breath-holding and using an ultra-low frequency force ballistocardiograph, all of the respiratory effect on the ballistocardiogram was eliminated. This can undoubtedly account for the fact that Malt and Harlan were unable to demonstrate significant ballistocardiographic changes in their patients during lipemia [*24*].

It is obvious that the minor degrees of arterial oxygen unsaturation observed in some hyperlipemic patients can contribute little to precipitate symptoms of myocardial and peripheral anoxia in these patients. The possibility that hyperlipemia may lower the arterial oxygen saturation by interfering with pulmonary oxygen diffusion in patients with mild congestive failure perhaps suggests that a high plasma triglyceride content may act in a similar manner to interfere with the diffusion of oxygen from the blood to tissue that is supplied by a diseased vessel.

An increase in the coagulability of the blood [*25*], sludging of the circulating red cells [*26*], increase in the viscosity, agglutination, adhesiveness and aggregation of the cells [*3,27*], and streaming of the chylomicrons [*28*] have been observed by a number of investigators, both in patients and in laboratory experiments on dogs, during the state of lipemia. Any one or a combination of these factors would adequately explain the effect of lipemia observed in these patients.

By alternating the Mellinkoff-Machella-Reinhold "fat-free" feeding with a full or high fat diet, we have been able in relatively short periods of time to induce marked changes in the blood lipids of these patients. By adopting this technic we found it possible to make more definitive correlative studies on lipemia and its effect on the circulation than we had previously found possible by the technic of fat feeding and administration of heparin in arteriosclerotic subjects [*27,28*]. The present investigation adds support to the observation that lipemia may aggravate arterial insufficiency in atherosclerotic patients [*1–5*].

SUMMARY

1. Rapid changes in the blood lipid levels were induced by manipulation of the dietary fat intake in six patients with essential hyperlipemia and clinical atherosclerosis. Studies were then made to correlate the changing blood lipid values with subjective and objective findings of cardiac and peripheral ischemia in each of these patients.

2. The patients' symptoms and signs of arterial ischemia showed prompt improvement with clearing of the lipemia. Reinstitution of high fat feeding for seven to twenty-one days returned all the patients to the state of hyperlipemia and to the same clinical status of their control or full diet period. It was found that in a given patient, manifestations of arterial insufficiency could be correlated, although roughly, with the rise and fall of the serum triglyceride level.

3. Evidence was found to indicate that hyperlipemia may, in certain patients, affect the oxygenation of arterial blood. It is suggested that this effects of lipemia in the coronary and peripheral arteries might interfere with the diffusion of an adequate amount of oxygen from the blood to meet the requirement of the tissues that are supplied by a diseased vessel.

4. The data reported in this present investigation add confirmative evidence to earlier observations that lipemia may be one of the factors that aggravates or precipitates acute arterial insufficiency in arteriosclerotic subjects. Satisfactory control of lipemia is therefore indicated in patients with severe coronary and peripheral vascular diseases.

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The Influence of Anticoagulant Therapy on the Incidence of Thromboembolism, Hemorrhage and Cardiac Rupture in Acute Myocardial Infarction*

Correlation of Clinical and Autopsy Data in 100 Cases

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THIS study was prompted by disagreement among different observers concerning the value and hazards of anticoagulant therapy in acute cardiac infarction [*1*]. Because it is sometimes difficult to make an accurate clinical diagnosis, both of the episode itself and of various complications, correlation of antemortem and autopsy data offers the only feasible method for clarifying certain relevant relationships. Interpretation of the results of such an analysis must be qualified by a number of limiting factors which will be considered in due course.

MATERIAL AND PROCEDURE

Analysis was made of the clinical records and autopsy protocols of 100 patients with acute myocardial infarction who died on the Medical Services of the Presbyterian and St. Luke's Hospitals during the period 1946 to 1953 inclusive.‡ Of these 100 subjects, fifty received anticoagulant therapy with heparin and dicumarol, and fifty were given comparable general treatment but no anticoagulants. The record of any patient who died during the first twenty-four hours after admission to the hospital was discarded. Complete bedrest was enforced in every instance during the early stages of illness. No case was included in the treated group unless therapy was deemed to have been *adequate*, according to the standards of the laboratory.

The Link-Shapiro modification of the Quick test, using whole plasma, was employed to determine prothrombin time.§ With a normal control of fourteen to sixteen seconds, the therapeutic range was considered to be between twenty-two and thirty-five. If the level fell below twenty-two seconds for more than twenty-four hours during dicumarol administration, heparin was added until adjustment upward was accomplished; if it rose above thirty-five, dicumarol was temporarily omitted and vitamin K or K_1 was administered, if necessary. When heparin was used, the venous clotting time was maintained between twenty-five and forty minutes (Lee-White method); if it exceeded the higher figure, the dosage was reduced.

In twenty-nine cases both heparin and dicumarol were employed; in eighteen, dicumarol alone; in three, heparin alone. Desired levels of effect were achieved in fourteen patients within the first three days following the estimated date of occurrence of infarction; in thirty, between the fourth and seventh days; in six, during the second week.

Autopsy protocols were chosen for review without reference to any factor other than the presence of recent cardiac infarction; this was the determining feature. Postmortem examinations were made in the routine manner; cases in which the heart only was

‡ The preponderant majority were obtained from the Presbyterian Hospital. Some of these cases have been included in clinical studies made at both institutions, and previously reported [*2,3*].

§ In some of the cases from St. Luke's Hospital the same test, using 12.5 per cent dilute plasma, was also made.

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examined were excluded. The brain usually was not removed except when there was clinical evidence of a cerebral lesion and, with rare exceptions, a search was not made for thrombi in the veins of the extremities. The viscera were examined microscopically in every instance.

The rigid criteria for acceptance which were arbitrarily adopted and the relatively small proportion of autopsies obtainable made it difficult to assemble a large series; it was for this reason that material from two hospitals, both affiliated with Columbia University, was combined. It is realized that there are numerous sources of bias in selection and presentation, applicable alike to treated and untreated groups; but it was not practicable to take into account all of the many variables involved.

TABLE I

INCIDENCE OF COMPLICATIONS AND ACCURACY OF CLINICAL DIAGNOSES, AS GAUGED BY AUTOPSY FINDINGS (100 CASES)

Complication	Not Treated (50)		Treated (50)	
	Found at Autopsy	Not Recognized Clinically	Found at Autopsy	Not Recognized Clinically
Mural thrombi	21 (42%)	16 (76%)	22 (44%)	19 (86%)
Pulmonary emboli	5 (10%)	2 (40%)	6 (12%)	4 (67%)
Systemic emboli	6 (12%)	3 (50%)	9 (18%)	6 (67%)
Major hemorrhage	0	0	6 (12%)	2 (33%)

RESULTS

Accuracy of Clinical Diagnosis. In selecting records from the files, a number of mistaken diagnoses were encountered. Among the fifty untreated patients, recent cardiac infarction was not recognized antemortem in ten instances, an error of 20 per cent. In the course of choosing fifty cases for inclusion in the treated group, eight were found in which the clinical diagnosis of infarction was not confirmed at autopsy, an error of 14 per cent. The records of these eight patients, all of whom had received anticoagulant therapy, were discarded in the final analysis. However, they must be included in calculating the over-all diagnostic error which thus was 17 per cent.

In their review of the clinical and autopsy records of 500 patients who died in Barnes Hospital with acute cardiac infarction, Lee et al., in the Department of Pathology of Washington University, found that the correct diagnosis was not made during life in 19 per cent [*4*]. This figure closely approximates our own. Widely divergent results have been noted by others. For example, in eighty-nine autopsy protocols reviewed by Wright et al. only 2 per cent of clinical diagnoses were not confirmed [*5*]. However, in this particular series, cases were not included in which the diagnosis appeared doubtful to the clinician. By way of contrast, there is the report by Paton, of the Royal Infirmary of Edinburgh, on 266 cases observed during 1954 and 1955. Postmortem examinations revealed incorrect diagnoses in 56 per cent [*6*].

Thromboembolism (Table I). In the fifty cases not treated, autopsy showed mural thrombi in the heart in twenty-one; in sixteen of these the diagnosis was not made antemortem. In only one instance was an intracardiac thrombus, without manifest embolization, recognized clinically. In the fifty cases treated, mural thrombi were present in the heart at autopsy in twenty-two; in nineteen of these the correct clinical diagnosis was not made.

In the fifty cases not treated, pulmonary emboli were found at autopsy in five; in two of these the diagnosis was not made antemortem. In the fifty treated cases, emboli were found in the lungs at autopsy in six; in four of these the diagnosis was not made antemortem.

In the fifty cases not treated, systemic emboli were found at autopsy in six; in three of these the diagnosis was not made antemortem. In the fifty treated cases, peripheral emboli were found at autopsy in nine; in six of these the diagnosis was not made antemortem. All of these emboli occurred in patients with mural thrombi in the heart, with one exception. This was a man in the untreated group who had had atrial fibrillation for many years and died following embolization to the left femoral artery. There was no mural thrombus adjacent to the infarcted area but thrombi were present in both atria.

It is thus apparent that, in this series, thromboembolism was found at autopsy in thirty-two of the untreated patients and in thirty-seven of those treated. To be sure, the numbers involved are small, but the figures show a balance in favor of the untreated. Clinically, these complications were not recognized in twenty-one of the untreated and twenty-nine of the treated group; perhaps the difference may be regarded as an example of unconscious bias on the part of the observers.

In a series of 312 patients who died with

cardiac infarction at the Massachusetts General Hospital during the years 1946 to 1951, postmortem examination showed that mural thrombi in the heart were not prevented by adequate anticoagulant therapy, for they occurred at the same rate in the treated and untreated groups [7]. In a similar series of 222 patients at Barnes Hospital, in the period 1946 to 1954, evidence of thromboembolism was noted at autopsy in 68 per cent of the untreated, in 78 per cent of those in whom treatment was initiated three days after occurrence of the acute attack, and in 45 per cent of those who received adequate therapy begun within three days after clinical onset. The conclusion was reached that treatment is not effective unless started during the first three days of illness [8]. Our own observations on this point are too few to justify an opinion.

Varying views have been expressed abroad. In Edinburgh, Gilchrist and Tulloch, on the basis of 248 postmortem examinations performed between 1947 and 1954, deny the claim that anticoagulants are responsible for a significant reduction in the incidence of intraventricular thrombosis or of systemic emboli [9]. Because there were fewer instances of pulmonary infarction in their treated cases, they emphasize the importance of the peripheral venous system as a source of emboli; in their opinion it is the effect of anticoagulants on this area that justifies their use in acute myocardial infarction. A comparable conclusion was reached by Honey and Truelove, who reviewed the autopsy reports of 136 patients with acute cardiac infarction who died in the Radcliff Infirmary, Oxford, during the period 1940 to 1954 [10]. They were impressed by the fact that the use of anticoagulants was accompanied by a striking reduction in the number of fatalities due to pulmonary embolism; in other respects the immediate causes of death were essentially the same in treated and untreated patients.

In our own series there was no important difference in the incidence of fatal thromboembolism in the two groups; it was thought that death could be attributed to this cause in five of the untreated and seven of the treated patients.

Major Extracardiac Hemorrhage (Table I). By major hemorrhage is meant one which, due to its size or location, jeopardizes the life of the patient or is the main factor in causing death. In the fifty untreated patients such hemorrhage was not observed, either clinically or at autopsy. In the fifty who received anticoagulants, serious hemorrhage outside of the heart was found at autopsy in six cases (12 per cent), but was recognized antemortem in only two of these. In three instances bleeding originated in the urinary tract, in two, cerebral hemorrhage occurred, and in one there was extensive bleeding into the pancreas and peritoneal cavity. Hemopericardum, without cardiac rupture, although noted by others, was not seen in any of our cases.

In the two patients with intracranial bleeding, and in the one with pancreatitis and bloody peritoneal fluid, hemorrhage was regarded as the chief cause of death. In the autopsy series of Wright et al. [5] extracardiac hemorrhage was not considered to be the immediate or contributing cause of death in any patient in the treated group. However, excluding cases with cardiac rupture, one untreated patient and three who were treated died with intracardiac hemorrhage (usually hemopericardium), to which the fatal outcome was attributed.

Subintimal Hemorrhage. In five of our cases the pathologist ascribed occlusion of the coronary artery to hemorrhage occurring beneath an atherosclerotic plaque. Four of these cases were in the treated group and one in the untreated. It was not possible to associate such hemorrhage with therapy or to determine whether anticoagulants had tended to prolong bleeding.

Congestive Heart Failure. It has been suggested that thromboembolism is more likely to occur in the presence of heart failure. According to accepted criteria, failure was present in thirty-eight of the untreated and forty of the treated patients. Its presence does not appear to have exerted any material influence on the incidence of thromboembolism.

Cardiac Rupture (Table II). The number of examples of rupture of the heart in our series was small. In the untreated cases it occurred only once; in the treated it was noted three times. To delineate a more satisfactory picture of the comparative incidence of this complication, a composite was made by combining our 100 cases with three American series, already reported, in which autopsies were obtained: eighty-nine cases assembled for the American Heart Association Study [5], 312 from the Massachusetts General Hospital [7] and 395 from Barnes Hospital [4]. Among the patients who received anticoagulants, only those have been included in whom treatment was considered to have been adequate, according to the statements of the authors. In all, the group comprises 796 cases—

TABLE II
INCIDENCE OF CARDIAC RUPTURE IN 557 UNTREATED AND 239 ADEQUATELY TREATED PATIENTS, BASED ON PRESENT SERIES AND THREE PREVIOUSLY REPORTED

Source	Not Treated			Treated		
	Total No. of Cases	Rupture		Total No. of Cases	Rupture	
		No.	Per cent		No.	Per cent
Wright et al. [5]	48	4	8.3	41	8	19.5
Waldron et al. [7]	241	12	5.0	71	10	14.1
Lee and O'Neal [8]	218	5	2.3	77	8	10.0
Authors' series	50	1	2.0	50	3	6.0
Totals	557	22	3.9	239	29	12.1

557 not treated and 239 treated. In the untreated, rupture occurred in 3.9 per cent; in the treated, it was noted in 12.1 per cent. Rupture, then, was found more than three times as often in the group that received anticoagulants; and it is of particular interest that this ratio was fairly constant in the individual series.

COMMENTS

This study represents a factual presentation of the material at our disposal, for we are fully aware of the danger of attempting to draw unbiased conclusions based on the clinical records and autopsy protocols of a hospital population. Although apparently this fallacy is not well known to medical investigators, it has been amply demonstrated by statisticians [*11–13*]. Hospital patients are not representative of the general population of sick persons and the pathologist's sample is determined by factors over which he has no control.

In discussing bias in postmortem statistics, Colin White wrote: "Whenever an investigator is forced to draw his sample, not from the population in which he is interested but from some pre-selected portion of it, he is in danger of obtaining a biased group. In effect there are two stages of sampling; the investigator controls the second stage, and perhaps carries out his work with scrupulous care; but this may be of no avail if there has been bias in the first stage." He further remarks that the material available to the pathologist "is necessarily obtained by methods of sampling which invalidate tests of significance that a statistician employs" [*12*].

The manner in which such a fallacy can occur in autopsy data has been exemplified and amplified by Mainland, who stated that "numerous factors besides fatality rates can produce the same effect, such as age, sex, race, socioeconomic level, disease incidence rates, the factors that determine admission to a hospital and those that cause selection of deceased patients for autopsy. Competition among these selection rates is very complex, and only a small part of the resulting bias can be measured or avoided.". He concluded by saying that "bias may mask a real association as well as create a fallacious one" [*13*]. In patients with coronary heart disease many other variables are inherent in the very nature of the disorder.

The limitations of the method are evident. Currently, however, no better approach is available which will disclose discrepancies between observations made at the bedside and conditions found at the autopsy table. The present analysis indicates trends which seem clearly defined.

SUMMARY

1. Analysis was made of the clinical records and autopsy protocols of 100 patients who died with acute myocardial infarction. Fifty were given adequate anticoagulant therapy; fifty did not receive anticoagulants.

2. Postmortem examinations revealed many errors in clinical diagnosis, both with respect to the presence of a recent cardiac infarct and of thromboembolic complications. At autopsy, such complications were found with equal frequency in treated and untreated groups. The incidence was not modified by the presence of congestive heart failure.

3. Major hemorrhage was observed in six of the treated cases; in three, death was attributed to this cause.

4. In 239 patients adequately treated and 557 untreated, assembled by combining this and three reported series, myocardial rutpure was found at autopsy three times as often in the treated as in the untreated.

5. Because numerous sources of bias are unavoidable when the records of a hospital population are used as the basis of a postmortem study, the results are not suitable for meaningful statistical analysis. However, only by a comparison with autopsy findings can the accuracy of clinical diagnoses be checked. The present study has demonstrated trends which seem definite.

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Reviews

Retinopathies Contrasted*

Diagnostic and Prognostic Significance of the Optic Fundi in Accelerated Hypertension

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SINCE many disorders which primarily or secondarily affect the vascular system may exhibit manifestations in the eyegrounds which appear superficially similar, it is important for the physician to recognize and distinguish those retinal lesions which show characteristic features. The glimpses afforded by funduscopic examination often are inadequate for this purpose due to the patient's inability to cooperate fully or to the physician's lack of experience with the ophthalmoscope. In addition, subtle changes may frequently escape notice since their detection depends largely upon the examiner's recollection of previous examinations.

Reflex color photography of the ocular fundus now facilitates a clearer delineation of the nature of many of the vascular retinopathies. In addition, serial photographs in the same patients can document the natural history, variability and reversibility of specific retinal lesions. Such observations when correlated with the clinical course of the disease help to clarify both the diagnostic and the prognostic importance of retinopathy.

Perhaps the most frequent utilization of funduscopy by the clinician has been made in studying patients with hypertension wherein the appearance of hemorrhage, exudate or papilledema in the eyegrounds is believed by many to suggest the advent of an accelerated phase of the disease. Similarly, in follow-up evaluation of hypertensive patients treated with depressor drugs, diets and surgical procedures, considerable significance has been assigned to the clearing of retinopathy which had been present previously.

For this presentation representative eyeground pictures taken during the course of a five-year study of hypertensive retinopathy have been selected along with examples of other types of retinal lesions from which differentiation must be made. The photographs have been chosen in order to demonstrate: (1) characteristic features of the retinopathies associated with accelerated hypertension, diabetes, leukemia, anemia, subdural and subarachnoid hemorrhage, renal and collagen diseases, (2) varying degrees of reversibility of the retinal changes associated with pheochromocytoma, hypertensive encephalopathy, chronic glomerulonephritis and other renal disorders, (3) the appearance of a persistent cuff of edema surrounding the optic disc as papilledema recedes, (4) disappearance of small caliber retinal vessels concomitant with progres-

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sion of renal disease, (5) progression and regression of hypertensive retinopathy with and without lowering of blood pressure level by antihypertensive agents.

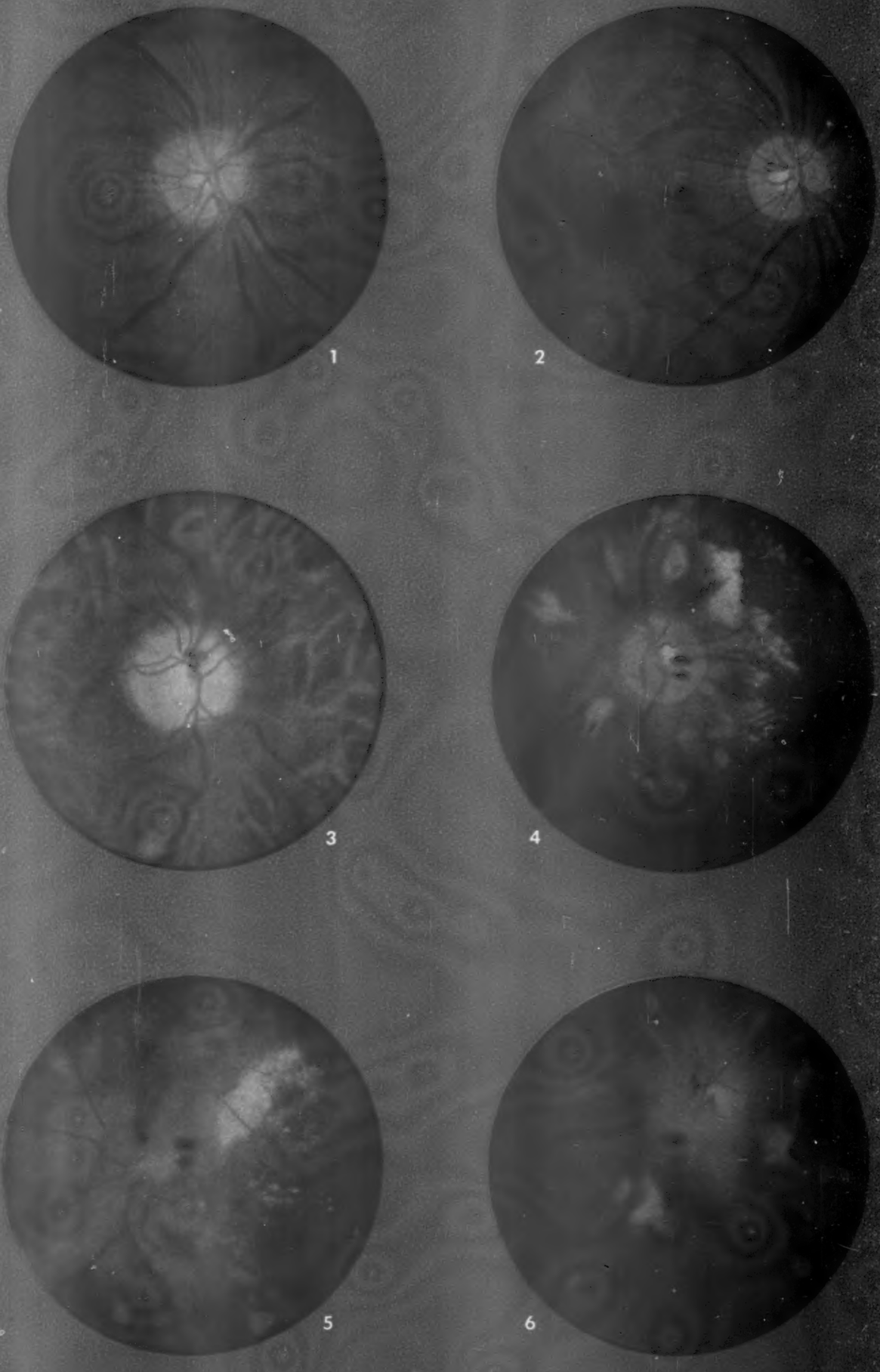
The photographs were made with a Bausch and Lomb retinal camera at a carbon arc intensity of 10 amperes and a shutter speed of $\frac{1}{15}$ of a second. Eastman Daylight Kodachrome film was used throughout. The two black dots which appear at the center of all photographs are an integral part of the ophthalmoscopic camera lens and serve to damp out light flares from the carbon arc. Two views of each fundus have been included in the photographic routine employed. In one the camera has been centered directly on the optic disc, in the other midway between the disc and the macula.

Significant anatomic features of the *normal optic fundus* are depicted in Figures 1 and 2, as seen in two views of the right fundus of a healthy twenty-eight year old Negro woman. In Figure 1 the camera has been centered directly on the red-orange optic disc through which the *central retinal artery* enters and promptly gives off a major superior and inferior branch, each of which divides into temporal and nasal branches. As the retinal arteries successively subdivide into smaller radicles they can be seen to give off their branches at approximately right angles. After the second branchings the arteries lose their muscular coats and properly may be termed arterioles. Eventually they become capillaries and extend more deeply into the retina. Corresponding in general distribution to the arteries are the broader, darker-colored veins whose smaller branches join to form larger channels which finally penetrate the optic disc and leave the fundus as the *central retinal vein.* In Figure 2 the camera has been centered on the temporal side of the optic disc, midway between the disc and the *macula lutea* with its central excavation, the *fovea centralis.* The latter, the point of greatest visual acuity, is seen here as a small light dot. The macula, located exactly at the posterior pole of the eye, subserves central vision, is devoid of visible blood vessels and appears somewhat darker than the surrounding fundus. The major fundal vessels course among the unmyelinated fibers of the *nerve fiber layer* (*stratum opticum*) of the retina. This layer lies just posterior to the *vitreous* and is separated from it by an interposed hyaline membrane, the *internal limiting membrane.* The nerve fibers, running parallel with the surface of the retina, traverse the fundus to converge at the optic disc and determine in large measure the shape, position and characteristic configuration of certain hemorrhagic and exudative retinopathies. In general the fibers radiate toward the disc like the spokes of a wheel converging at the hub. Fibers emanating from the macular region, however, are grouped into a roughly elliptical bundle with the disc and macula describing the major axis. This anatomic arrangement is likewise important as it figures prominently in defining the pattern of certain retinal lesions and in determining the course of migration of edema residues away from the disc.

Since the retina itself is a transparent membrane its background color is derived from the underlying choroidal vessels and the retinal layer of pigment-epithelium which lies just anterior to the choroid. Variation in pigmentary characteristics between races and even among individuals of the same race therefore influence the color of the fundus. In general, the fundi of persons of extremely fair complexion will be light orange in color (Fig. 14) while those of the Negro will be darker with more brown and gray tones. (Figs. 1 and 9.) Between these extremes many degrees of color variation may be observed. When the retinal pigment-epithelium layer is thin or poorly developed the vessels of the choroid become directly visible, appearing as a meshwork of broad orange-red channels freely anastomosing between more darkly pigmented intravascular spaces. Such fundi are called *tigroid* or *tessellated* fundi. (Figs. 3, 15 and 16.)

RETINAL HEMORRHAGE

When blood is extravasated into the nerve fiber layer of the retina it is free to extend longitudinally between the fiber bundles which are arranged in a plane parallel to the retinal surface. The long axis of such hemorrhage, therefore, follows the course of the nerve fibers, and appropriate descriptive terms such as *linear, elongated, streak* and *flame-shaped* have been ap-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plied. The margins of these hemorrhages show an irregular or feathered edge. When fresh their color is bright red, but with age they darken to brown. When there is partial obstruction to venous return through the central vein (as with papilledema), multiple hemorrhages of this type may closely surround the disc in radiating fashion.

HYPERTENSIVE HEMORRHAGES

The hemorrhages which are seen in association with advanced hypertension are classically those of the nerve fiber layer type and may be of arterial, venous or capillary origin. Examples are shown in Figures 3, 4, 5 and 6. Their configuration affords no clue to the underlying etiology of the elevated blood pressure; however, many observers believe that the mere appearance of hemorrhage suggests the acceleration of a previously benign hypertensive process. Other disorders causing nerve fiber layer hemorrhage of course may afflict the hypertensive patient and therefore must be excluded.

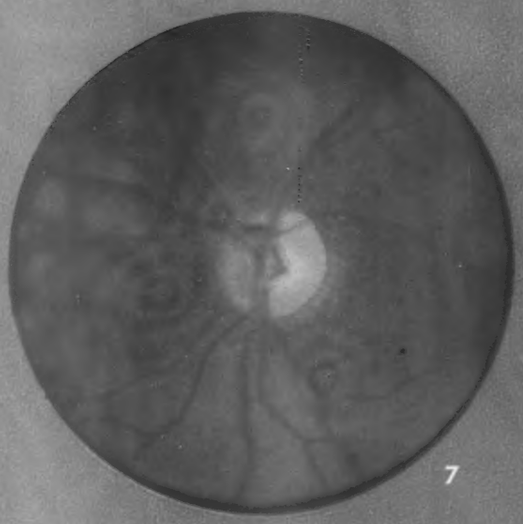
In accelerated hypertension hemorrhages may appear singly or in showers, and fresh ones often crop out while older ones are being resorbed. Most commonly they are located within two or three disc diameters of the nerve head, sparing the peripheral portions of the fundus. Although they occur in greater profusion when papilledema coexists, swelling of the nerve head is not a prerequisite for their appearance. In some instances the proximity of hemorrhage to a nearby vessel may define the site of origin as in Figure 3, in which a flame hemorrhage is seen above the disc in close relation to a dilated branch of the superior retinal vein.

DIABETIC HEMORRHAGES

Capillary aneurysms are perhaps the most consistent hallmark of the retinopathy associated with diabetes mellitus. Their appearance in the fundi can be correlated more closely with the duration of the diabetes than with the adequacy of control of glycosuria. Although dilatation of the retinal veins presumably antedates the development of aneurysms, such changes are sufficiently subtle that they frequently escape clinical notice. Seen first as scattered punctate red or red-purple dots (Fig. 7) the aneurysms later increase in size and number and may appear in lacy clusters particularly in the macular region. (Fig. 8.) When clearly visualized they are seen to consist of delicate vascular channels with discrete margins. Friedenwald and others have demonstrated that they represent saccular dilatations of the venous ends of capillaries with structural similarities to the renal lesions of diabetes. Because the aneurysms lie in the *inner nuclear layer* of the retina, deep to the major vessels and nerve fibers, the hemorrhages which originate from them appear initially as rounded or oval extravasations with sharply demarcated edges. With extensive leakage of blood more anterior structures may be invaded. In advanced stages of diabetes subhyaloid and vitreous hemorrhages may be observed. When the latter type of hemorrhage undergoes organization rather than resorption, new growth of delicate blood vessels and connective tissue occurs which may produce the striking retinal picture of *proliferating retinitis.* (Figs. 9 and 10.)

HEMORRHAGES OF LEUKEMIA AND ANEMIA

White-centered hemorrhages in the nerve fiber layer characterize the retinopathy of leukemia and many anemias but distinguishable differences do exist. In general, bleeding into the fundus in leukemia tends to produce larger, more massive hemorrhages with large white centers. (Figs. 11, 12 and 13.) Their appearance can be correlated more consistently with fall in the hemoglobin level than with thrombocytopenia. Restoration of hemoglobin results in resorption of the hemorrhage. Such manifestations are seen most frequently in chronic myelocytic and acute blastic forms of leukemia.



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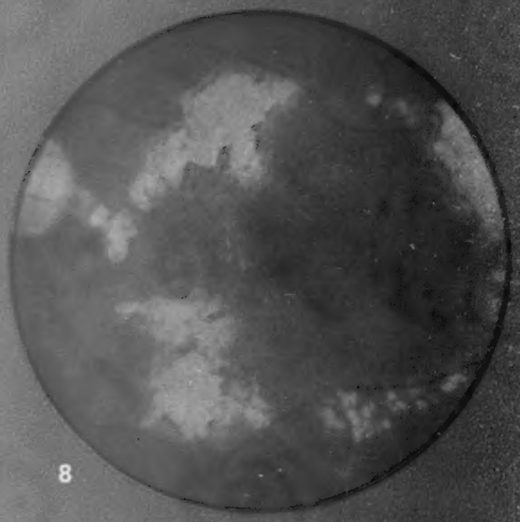

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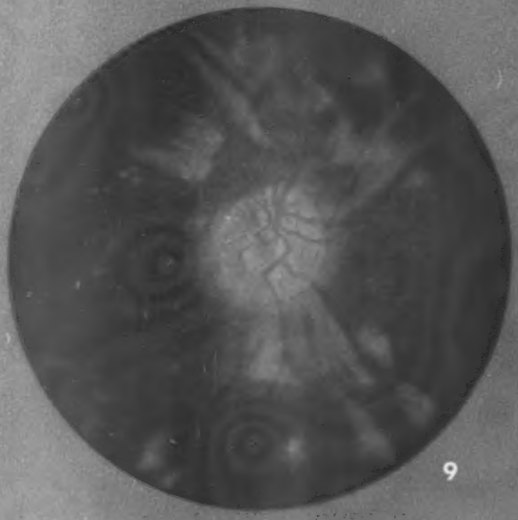

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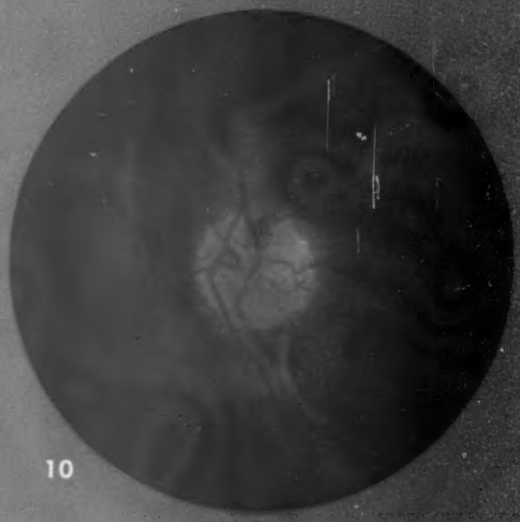

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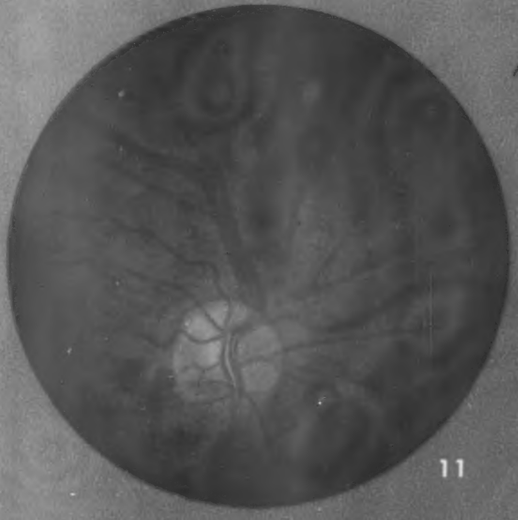

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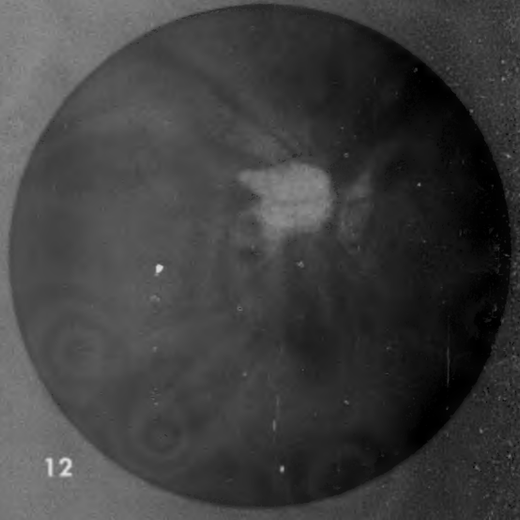

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Subhyaloid hemorrhages are frequently observed as well.

In anemia of any type when the hemoglobin level falls to the range of 5 gm. or less, white-centered hemorrhages may be observed in the fundi. Although these may assume moderate size in pernicious and nutritional megaloblastic anemia, in general they are smaller than those seen in the anemia of leukemia. Individual hemorrhages are oval or diamond-shaped with the long axis paralleling the path of the nerve fibers. Examples pictured are from a patient with nutritional megaloblastic anemia (Fig. 14) and one with acquired hemolytic anemia before (Fig. 15) and after (Fig. 16) replacement of hemoglobin.

The composition of the white centers remains in dispute. Some authorities contend that they comprise localized masses of degenerating leukocytes while others insist that they represent circumscribed patches of edema in the nerve fiber layer.

SUBHYALOID HEMORRHAGE

The trapping of blood between the nerve fiber layer and the hyaline membrane (*internal limiting membrane*) which separates it from the vitreous has been termed *subhyaloid* or *preretinal* hemorrhage. This may occur by direct extension of some of the retinal hemorrhages already described (as in diabetes or leukemia), as a result of trauma, or spontaneously without demonstrable cause. These hemorrhages appear shiny red in color and have smooth, rounded, sharply-defined borders. They vary in size from tiny blister-like bulges on the retinal surface to massive blebs obscuring a major portion of the fundus. An example of a large spontaneous subhyaloid hemorrhage is shown in Figure 17. In this unusual photograph, taken with the patient seated, the entire macular region is obliterated by a large hemorrhagic blister which shows at the top a serum level and at the bottom a brown crescent where the red cells have layered out. Four months later (Fig. 18), resorption of the hemorrhage was complete, with a small patch of depigmentation in the macular region as the only residual.

RETINAL HEMORRHAGES OCCURRING WITH SUBDURAL AND SUBARACHNOID BLEEDING

In association with intracranial bleeding of the subdural and subarachnoid type, retinal and preretinal hemorrhages of varying size and configuration may be seen. In all likelihood different mechanisms account for certain variations encountered, examples of which are illustrated in Figures 19–24. It must be recalled that reflections of both the dura mater and arachnoid membrane extend with the optic nerve within its sheath toward the posterior portion of the globe. Potential channels are thus provided by which blood can dissect directly from a bleeding point in the central nervous system along the optic sheath.

In Figure 19 the right fundus of a patient with traumatic subdural hemorrhage shows massive extravasation of blood surrounding and obscuring a swollen optic disc. The nerve fiber layer has been invaded since the hemorrhagic border is irregular and feathered in appearance.

Figure 20, the right fundus of a Negro woman with a leaking berry aneurysm, depicts a type of retinopathy observed most often with subarachnoid bleeding. Multiple round and oval hemorrhages with very dark centers and surrounded by light red halos are scattered throughout. Such hemorrhages, subhyaloid in position, occasionally may be seen to pop out in profusion during ophthalmoscopic examination of patients with acute subarachnoid or massive intracerebral bleeding. In this patient, papilledema and nerve fiber layer hemorrhage previously had been present, but by the time of photography these had disappeared.

The elderly hypertensive patient whose left (Fig. 21) and right (Fig. 22) fundi are pictured was recovering from her second bout of subarachnoid bleeding. Large round dark hemorrhages with fading pink halos wreath both discs. In Figure 22 the extravasation has invaded the nerve fiber layer and dissected toward the macula, producing an incomplete bloody macular star figure.

Figures 23 and 24 illustrate the gross pathologic conditions of the eye of a hypertensive patient with fatal massive cerebral hemorrhage whose retinas showed many round hemorrhages with dark centers and red halos. Viewed from

the side in Figure 23 are the globe and optic nerve from the central end of which hemorrhage can be seen dissecting along within the nerve sheath. Seen from the front through the intact lens (Fig. 24), multiple hemorrhages of the type described are observed directly.

EXUDATIVE CHANGES IN RENAL DISEASE AND HYPERTENSION

The varied exudative changes seen in the optic fundi represent different manifestations of the formation, migration, coalescence and resorption of retinal edema. In acute active forms of renal disease white fluffy conglomerates of edema frequently localize in the nerve fiber layer. These are commonly called *cotton wool patches;* they vary from a pinpoint up to a disc diameter in size, are irregular in shape and usually show furred or striated margins. They are readily resorbable, usually disappearing without residual in three to four weeks. Solitary patches frequently are situated at the bifurcations of retinal arteries. White patches may be seen about the optic disc in Figure 25, the right fundus of a thirty-six year old patient with active glomerulonephritis. Such lesions are indistinguishable from the so-called *cytoid bodies* which may appear in the fundus of a patient with collagen disease when renal involvement becomes severe.

In Figure 26, irregularly-shaped fluffy exudates conforming to the cytoid body lesions are seen in the left fundus of a patient with disseminated lupus erythematosus complicated by accelerated hypertension. Arterial attenuation and tortuosity and venous dilatation are noteworthy also.

Offering sharp contrast to the soft fluffiness of the cotton wool patches and cytoid bodies are the discrete yellow and white *punctate exudates* which frequently become widely distributed throughout the fundus in patients with chronic renal disease, especially in those with retinal vascular damage from hypertension. These scattered lesions lie deep to the major retinal vessels and although potentially evanescent, they disappear much more slowly than the cotton wool patches. Their less rapid resorption most likely reflects a more severe degree of retinal ischemia.

It must be remembered that transient edema residues of a highly mobile nature may be observed in the fundus when retinal edema and papilledema recede, regardless of the underlying disease present. Following the path described by the course of the nerve fibers, the edema fluid is dispersed peripherally from the disc region.

The diffuse scattering of exudate in Figure 27 has resulted from the migration and dispersion of residues from papilledema and retinal edema which were present previously. In this eighteen year old patient with disseminated lupus erythematosus exudates resembling cytoid bodies can be seen also, and peripapillary edema still obscures the disc margins. White, bloodless arterial branches may be observed at the right of the disc and smaller caliber retinal vessels are no longer visible.

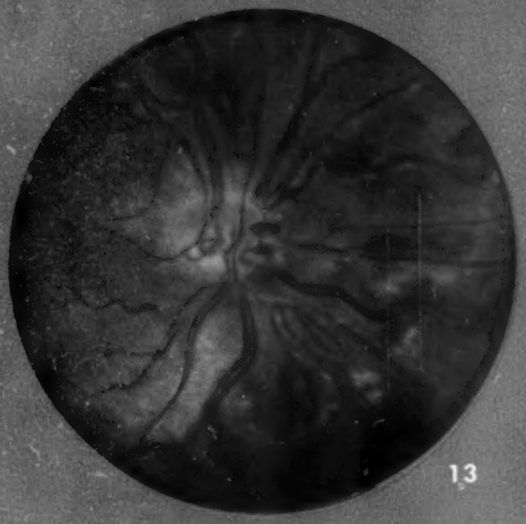
The *macular star figure* is perhaps the most striking of the many exudative manifestations seen in the retina and many observers have come to regard it as an ocular hallmark of renal disease. In Figure 28, the left fundus of a fifty year old uremic hypertensive patient with heart failure, a macular star is being formed. The clouded appearance of the fundus between macula and disc suggests the presence of retinal edema. Measurable papilledema had not been present in this patient.

Figure 29 shows the left fundus of a hypertensive patient with early uremia and heart failure whose papilledema receded with bedrest. As it cleared, edema fluid migrated toward the macula finally forming the fan-shaped figure which is limited to the nasal half of the macula.

A more complete stellate figure is illustrated in Figure 30, the right fundus of a forty year old Negro woman with chronic active pyelonephritis, hypertension and uremia. The arteries leaving the disc show marked sheathing and except for the macular region only scattered exudates remain above and below the disc.

EXUDATIVE CHANGES IN DIABETES

When nephropathy and hypertension complicate the course of diabetes mellitus the exudative



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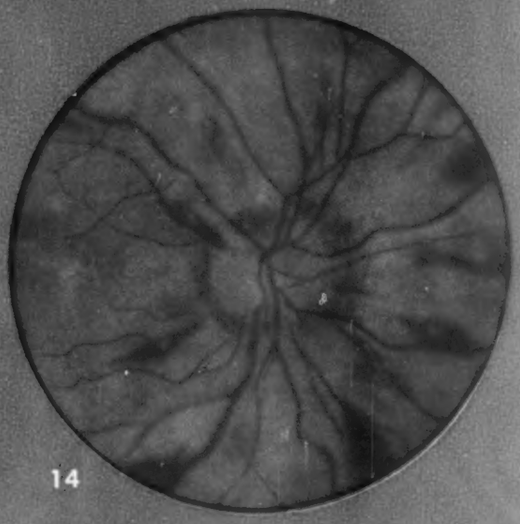

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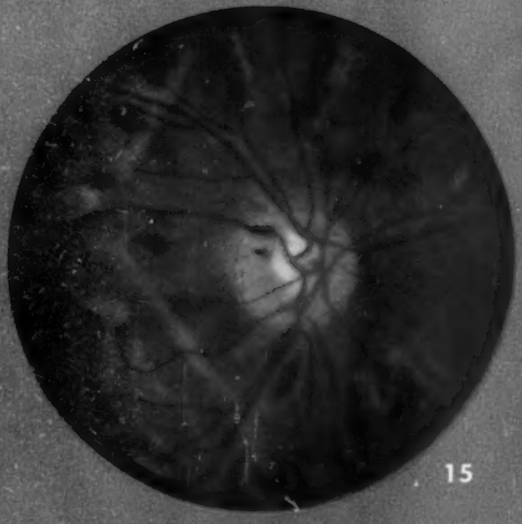

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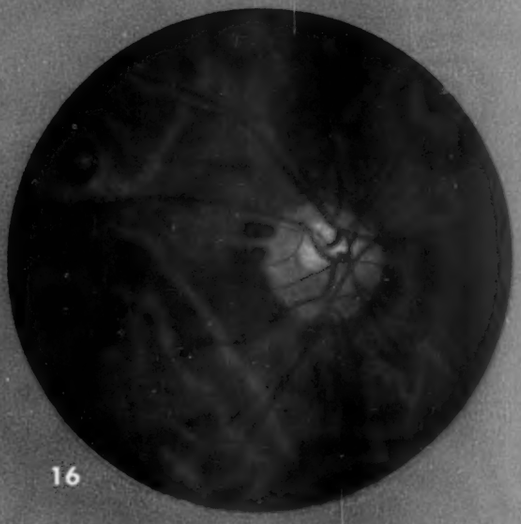

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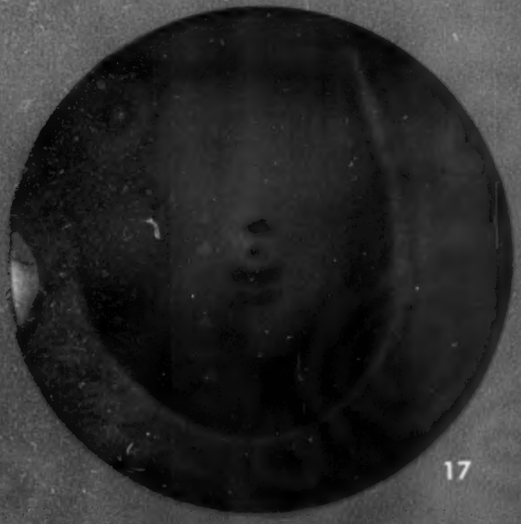

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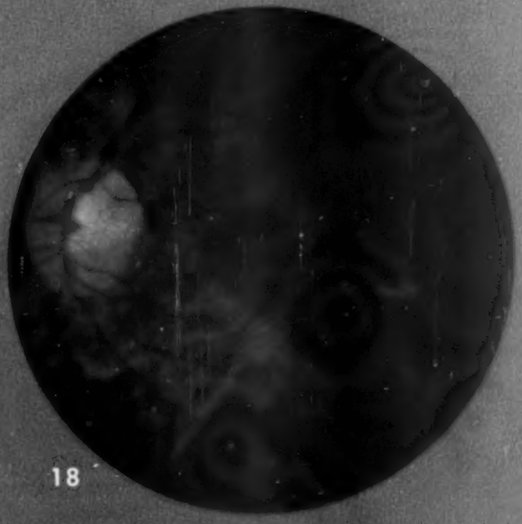

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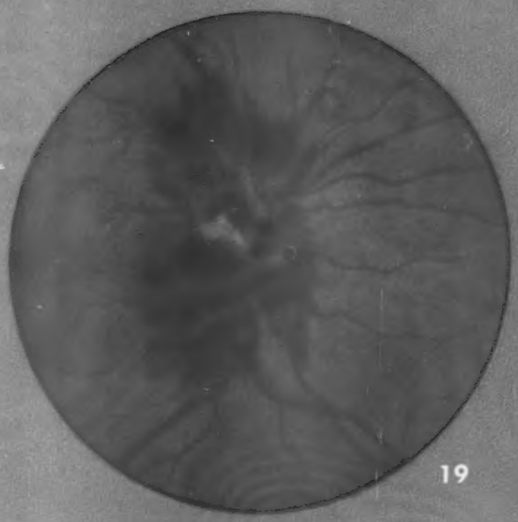

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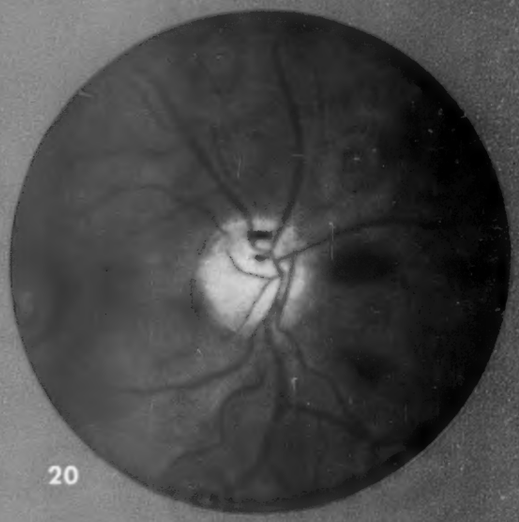

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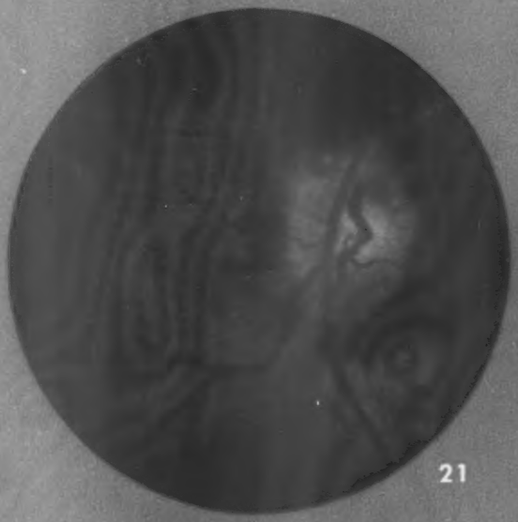

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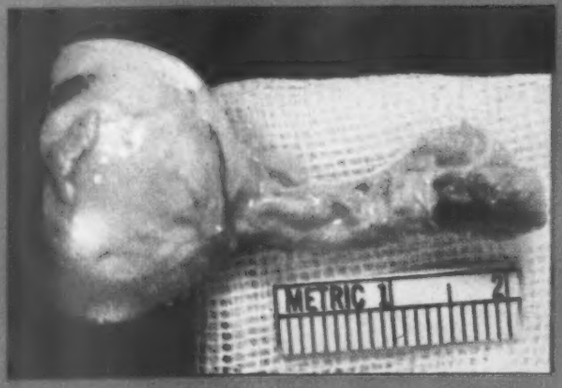

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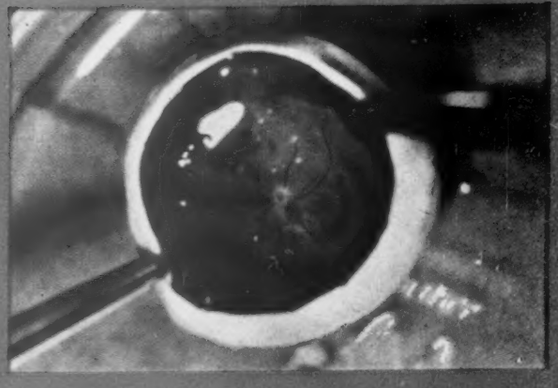

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changes of renal disease already described may be seen. Earlier in the course of the disease, however, prior to discernable renal involvement, the diabetic fundus may show scatterings of small yellow splashy exudates. These often appear shiny or waxy with sharp edges, and frequently they surround the macular region. Capillary aneurysms are almost always present in addition. In Figure 8 heavy sheets of hard yellow exudate may be seen forming a complete circle about the left macular region of a sixty-year old diabetic patient. This *circinate retinitis*, while suggestive of diabetes, is by no means pathognomonic of the disease.

When diabetes, hypertension and renal disease coexist in the same patient, features of all three retinopathies may be noted. Figure 9 illustrates such a combination in the right fundus of a thirty-three year old Negro woman with intercapillary glomerulosclerosis confirmed by renal biopsy.

HYPERTENSION AND PAPILLEDEMA

In the hypertensive patient papilledema (grade 4 fundi) has come to be regarded as the most consistently reliable sign that the disease has entered an accelerated phase. Implicit therein is the ominous prognosis of "malignant" hypertension. In this regard it must be remembered that papilledema frequently is seen in conditions in which hypertension may be merely an associated concomitant (acute glomerulonephritis, lead poisoning, head trauma, pseudotumor cerebri). In addition, certain complications which may befall the benign hypertensive patient can give rise to papilledema (spontaneous subarachnoid hemorrhage, leaking berry aneurysm, intracerebral hemorrhage). Obviously the success of management of the causative episode will be a better guide to prognosis in such instances than will the mere presence of papilledema.

When swelling of the optic disc occurs as a manifestation of the course of hypertension only minimal signs and symptoms may be produced until the process becomes fully developed. Loss of physiologic cupping, blurring of disc margins and overfilling of the veins must be sought carefully if the diagnosis is to be made at an early stage. It is generally agreed that the temporal margin of the disc is the last portion to become blurred.

Once papilledema has become well established it is not likely to escape detection at ophthalmoscopic examination although many striking variations may be observed. (Figs. 31–36.)

All details of the disc have been lost in Figure 31, the right fundus of a thirty-three year old white man with fulminant malignant essential hypertension, whose renal function had not yet failed at the time of photography. The veins are overfilled, and edema, exudates and streak hemorrhages obscure the peripapillary region. In spite of depressor drug therapy the disease progressed to death in uremia within a month.

Figure 32 shows marked papilledema in the left fundus of a thirty-two year old hypertensive Negro woman who was admitted with a two-week history of headache and progressive blindness. Multiple streak hemorrhages and heavy yellow exudate surround the greatly swollen optic disc. The urine contained innumerable red cells, and lumbar puncture revealed the spinal fluid pressure to be 500 mm. of water. The blood urea nitrogen of 40 mg. per cent rose progressively.

Figure 33, the right fundus of a thirty-seven year old man, depicts a receding phase of papilledema. This patient's papilledema had been present for two months and peripapillary edema residues are seen migrating toward the macula. Indistinct margins of the still enlarged disc are accentuated by a greyish halo. The latter phenomenon, an indication of regression of papilledema, will be illustrated further in later photographs.

Headaches, weakness and blurred vision were the complaints on admission of the forty-four year old hypertensive Negro whose right fundus is seen in Figure 34. Streak hemorrhages and yellow punctate exudates can be noted as well as edema residues streaming toward the macula. Although the blood urea nitrogen was normal on admission, treatment with ganglion blocking agents was to no avail and he died in uremia in five weeks.

Figures 35 and 36 show the right and left fundi, respectively, of two Negro patients with chronic renal disease, heart failure, accelerated hypertension and uremia. Both had massive

albuminuria. Chronic pyelonephritis was the underlying disease in the first patient and chronic glomerulonephritis in the second. In both instances the optic disc shows a "mushroom" type of papilledema and small caliber retinal vessels cannot be visualized. A nearly complete macular star is present in Figure 36.

THE POSTPAPILLEDEMA HALO

When papilledema has subsided its previous presence and extent sometimes can be suspected when ophthalmoscopic examination reveals a greyish halo surrounding the optic disc. This has been thought to represent a cuff of unresorbed edema fluid, but in some instances the grey ring may be noted for as long as a year after the disappearance of papilledema. If peripapillary exudates persist after subsidence of actual nerve head swelling, the halo is rather sharply set off from the surrounding fundus and the disc appears to float.

In Figures 37 and 38 this phenomenon is seen in the right fundi of two patients with accelerated hypertension and uremia secondary to chronic glomerulonephritis. Irregularity of the disc outlines suggests that some swelling is still present.

Figures 39 and 40 document the development of the halo in a twenty-five year old patient whose accelerated hypertensive syndrome was reversed by the removal of a pheochromocytoma. In Figure 39, preoperatively the left optic disc is massively swollen and a heavy blanket of exudate obscured the macula. In Figure 40, two months after surgical removal of the tumor and restoration of blood pressure to normal, a grey ring surrounds the disc as the macular exudates dissipate.

Two phases of receding papilledema are pictured in Figures 41 and 42, views of the left fundus of a forty-eight year old Negro with accelerated hypertension secondary to chronic renal disease. Figure 41 shows a few soft exudates, overfilled retinal veins and early shrinkage of the swollen disc with the appearance of the characteristic grey ring. In Figure 42, taken one month later, the ring is more prominent, the disc is smaller with more discrete margins, and the veins have resumed a more normal caliber.

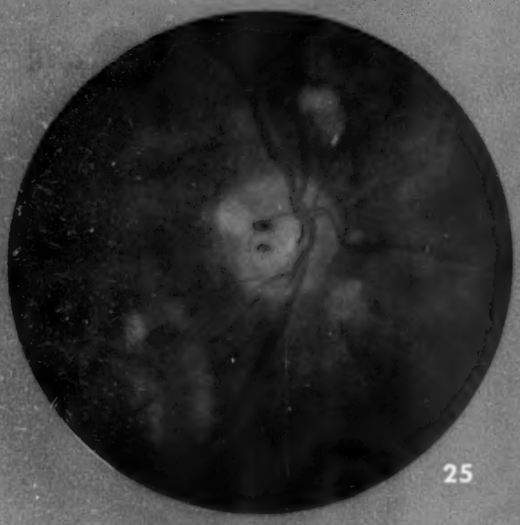
RETINOPATHY AND BLOOD PRESSURE LEVEL

Although high levels of diastolic pressure usually go hand in hand with the more severe grades of hypertensive retinopathy (grade 3 and grade 4), there has been no satisfactory demonstration that blood pressure height *per se* is responsible for the retinal changes, except as it may reflect the severity of the underlying vascular disturbance. Indeed, advanced retinopathy sometimes may be absent in patients with extremely high diastolic levels and, conversely, severe retinal changes occasionally occur when the diastolic pressure is only slightly or moderately elevated. To be sure, such instances represent exceptions to the general rule, but they emphasize the need for a clearer understanding of the natural history and reversibility of hypertensive retinopathy with respect to changes in blood pressure level. Since retinal ischemia plays a major role in the production of the more severe retinal manifestations, the distribution of the retinal vessels, the integrity of their lumens, and the elasticity and permeability of their walls must be reckoned with along with hemodynamic considerations.

REVERSIBILITY OF RETINOPATHY IN PHEOCHROMOCYTOMA

An unusual opportunity to observe the reversibility of retinopathy was afforded by a twenty-five year old white woman whose hypertension was abolished by surgical removal of a pheochromocytoma. Figures 43–48 illustrate the serial changes as noted in the patient's left fundus.

Referred for evaluation of fluctuant hypertension two months after her first pregnancy had been terminated for presumed pre-eclampsia, she complained of headache, nervousness, weakness and sweating spells. The blood pressure ranged from 100/70 to 230/150 mm. Hg, and she had pronounced postural hypotension and prominent clinical evidence of hypermetabolism. When first seen her left fundus (Fig. 43) showed marked papilledema, occasional hemorrhages and numerous soft cotton wool patches. Although her blood pressure level was reasonably



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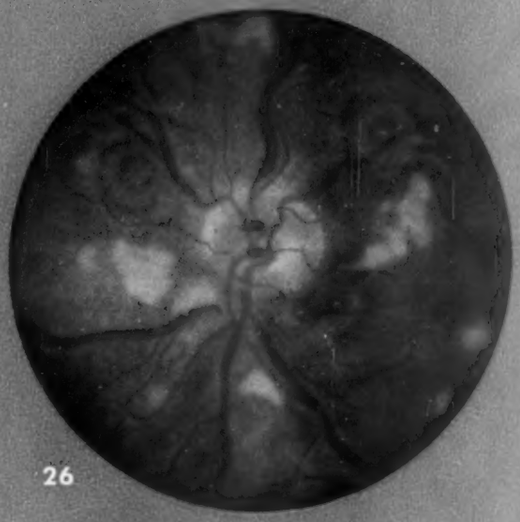

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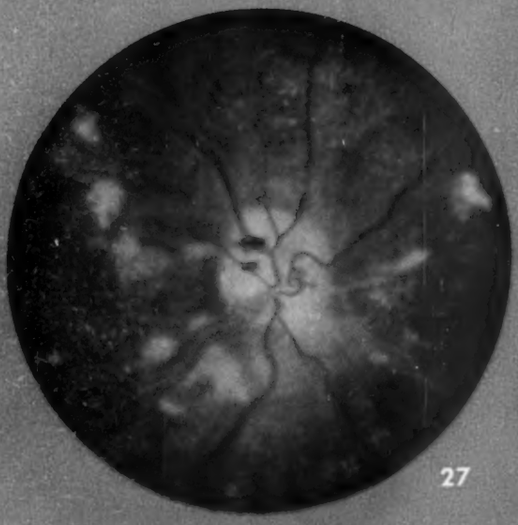

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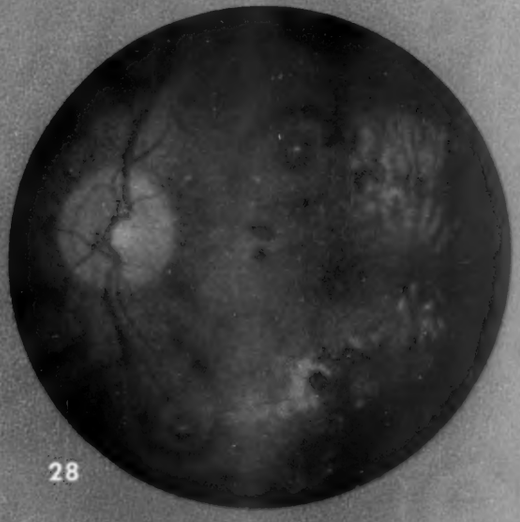

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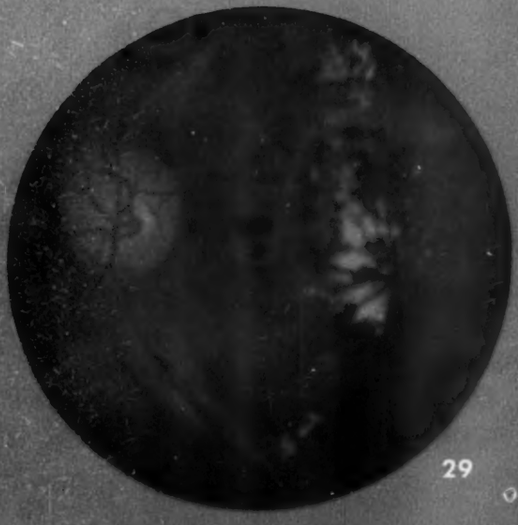

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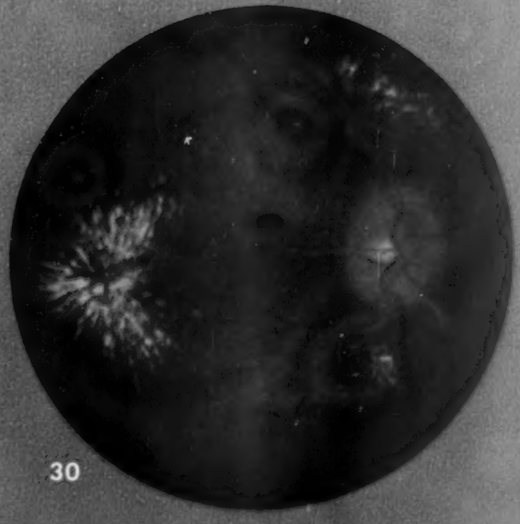

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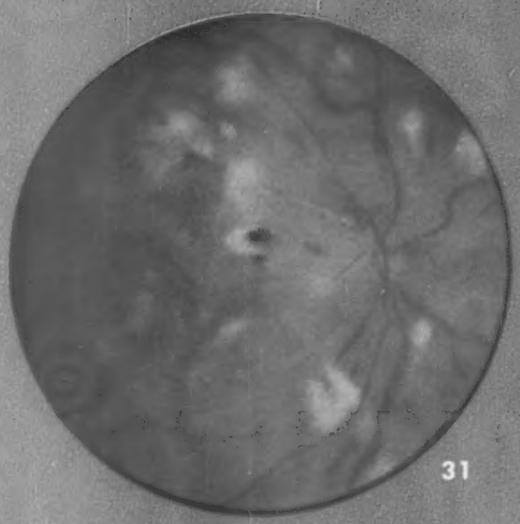

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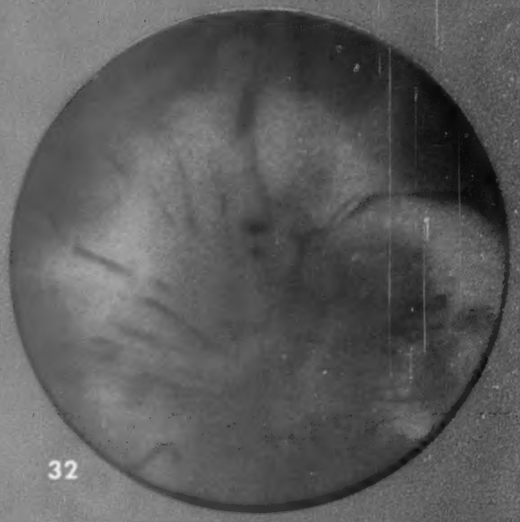

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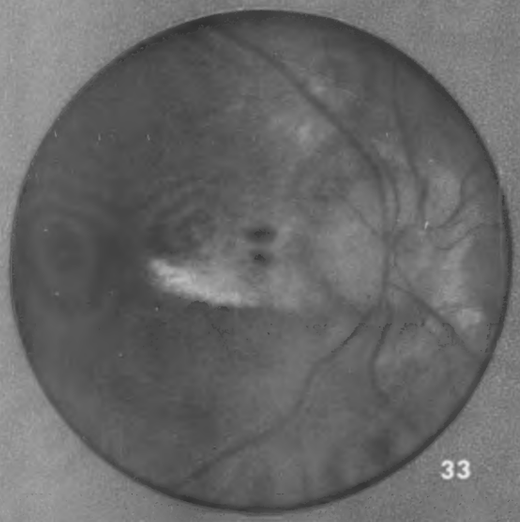

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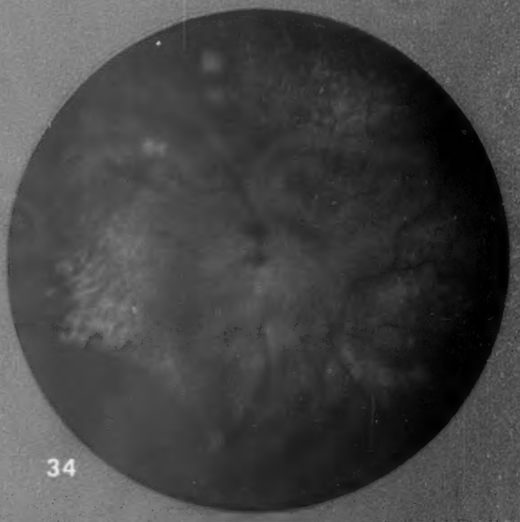

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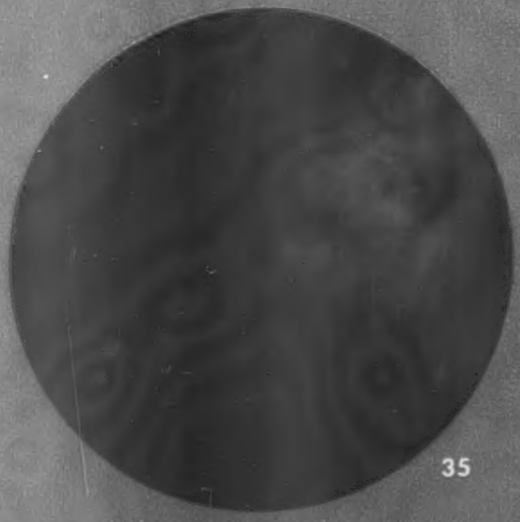

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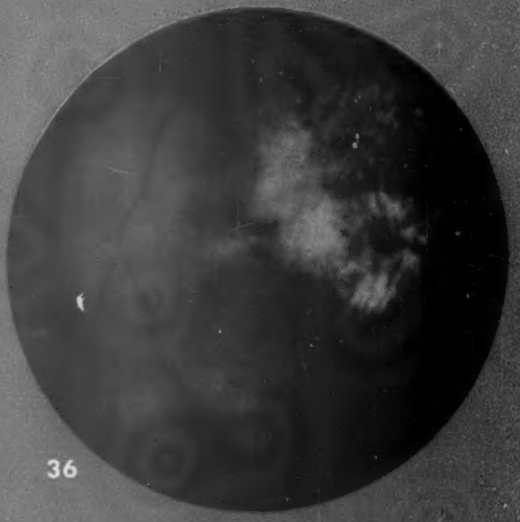

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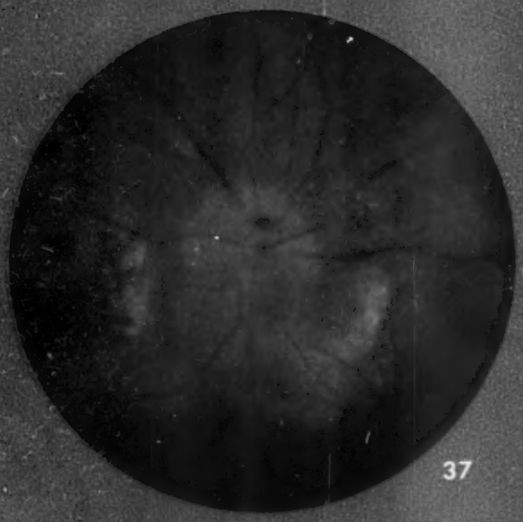

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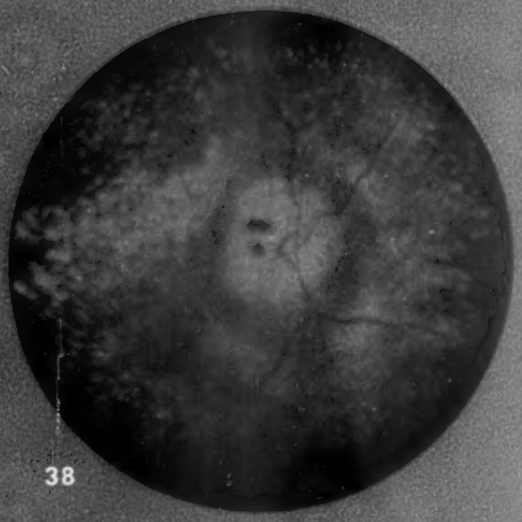

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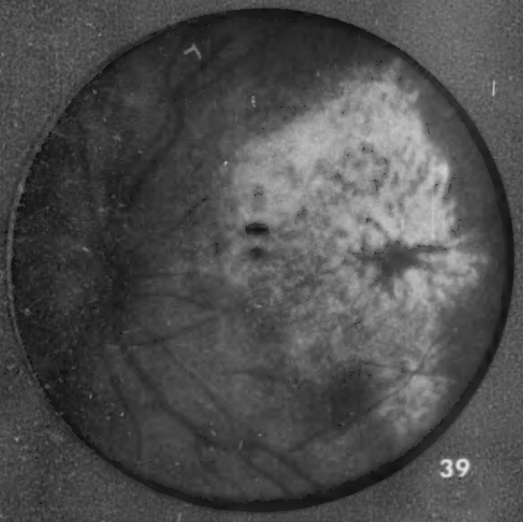

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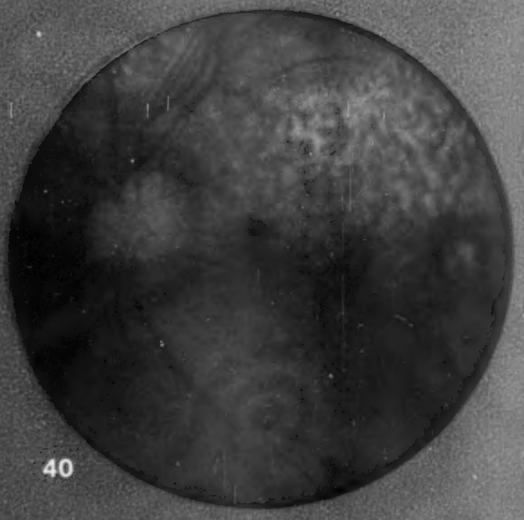

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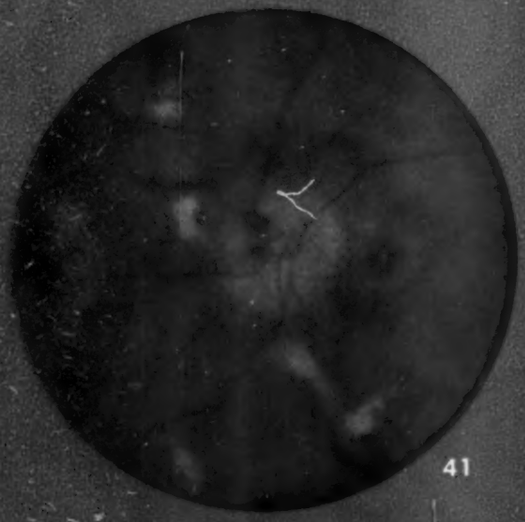

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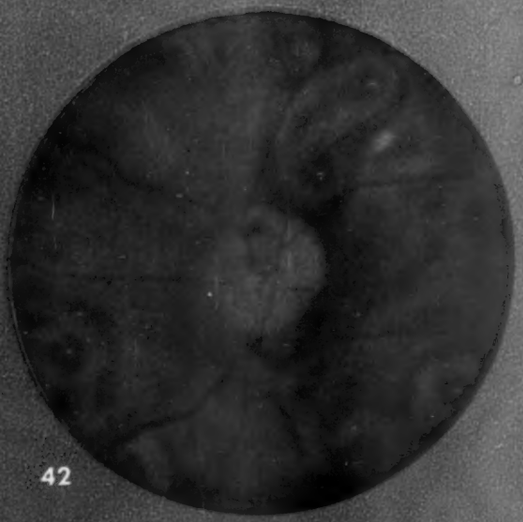

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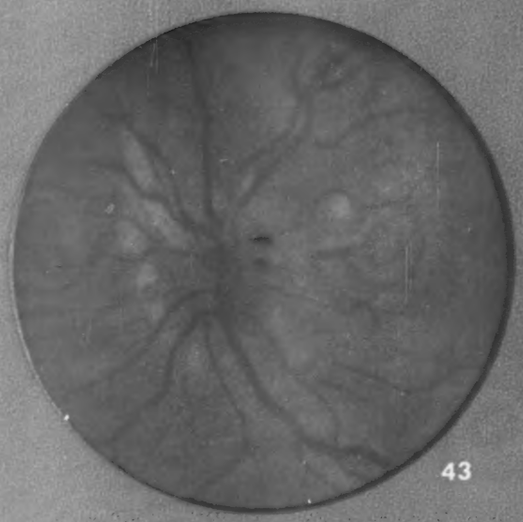

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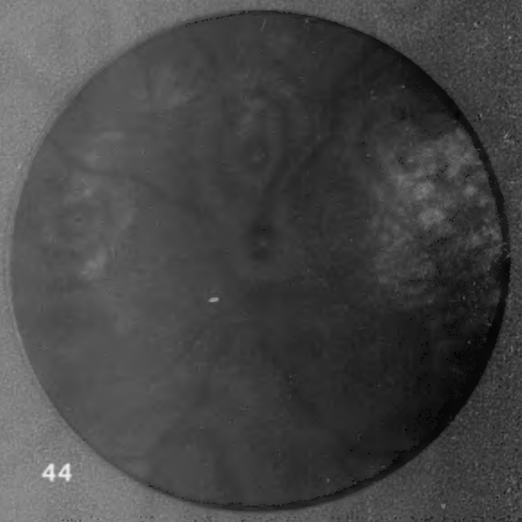

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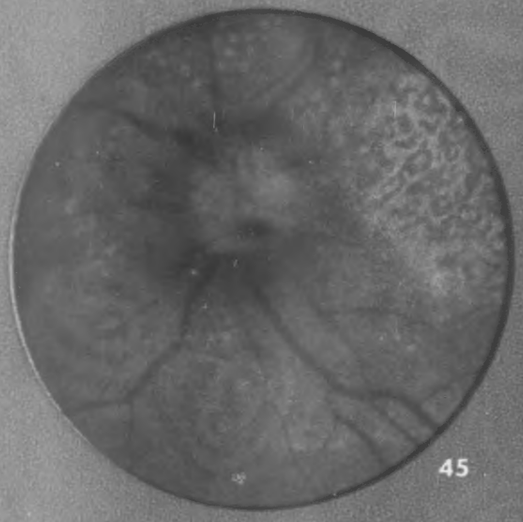

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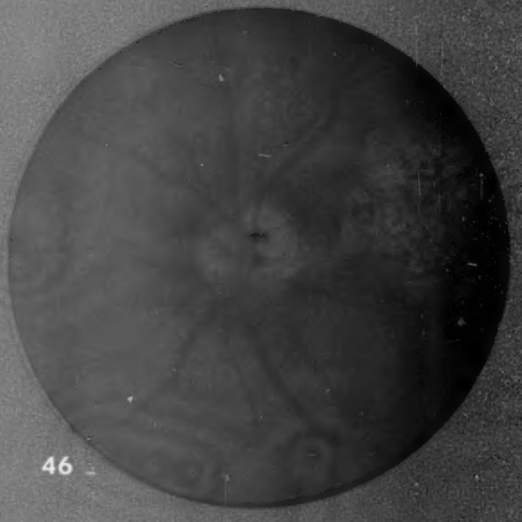

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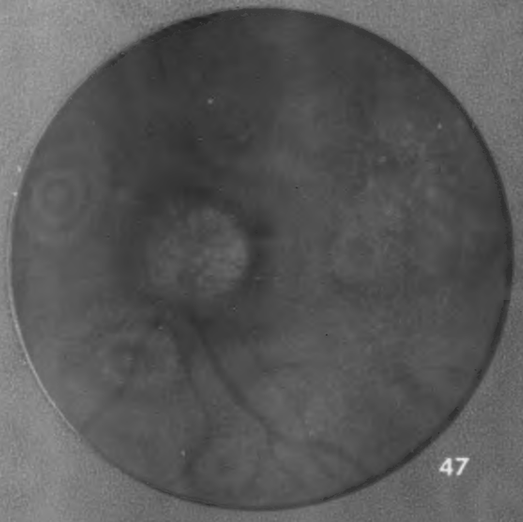

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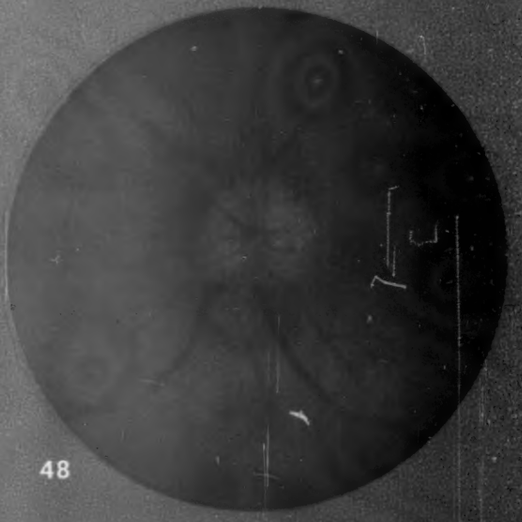

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well controlled with antiadrenergic drugs, during an unavoidably prolonged preoperative period (two months) the retinopathy worsened and visual acuity was moderately affected. In Figure 44, taken four weeks after resection of a 100-gm. tumor and restoration of blood pressure to normal, papilledema is still present and heavy macular exudates have been laid down. Many of the smaller retinal vessels are completely obscured. Figure 45 shows the appearance of the fundus ten weeks after surgery. The disc has resumed more normal size and a prominent halo is present. Unresorbed exudates remain. Progressive dissipation of the exudates with prolonged persistence of the postpapilledema halo is illustrated in Figures 46, 47 and 48, four months, seven months and ten months, respectively, after surgery.

REVERSIBILITY OF RETINOPATHY IN CHRONIC GLOMERULONEPHRITIS

Even after moderate renal insufficiency develops in chronic glomerulonephritis the further course frequently may be a protracted one. In such patients observation of serial retinal changes with relation to other clinical and laboratory manifestations often yields correlations which emphasize that factors other than blood pressure level also are important in the production of neuroretinopathy.

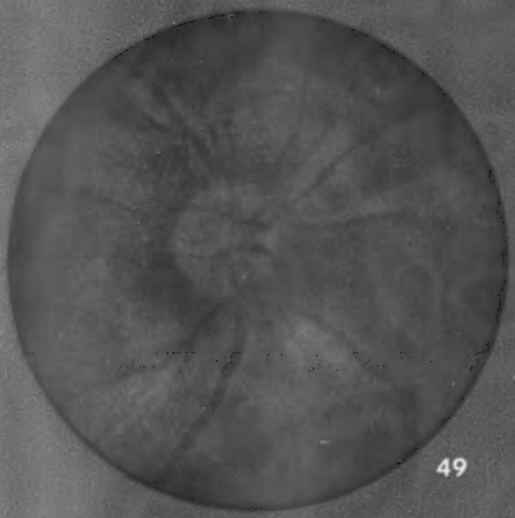
The thirty-seven year old Negro woman whose right fundal changes are pictured serially in Figures 49–54 was followed through three separate bouts of grade 4 retinopathy over a three year period. She was initially thought to have accelerated essential hypertension; the correct diagnosis (chronic glomerulonephritis) was subsequently established by renal biopsy. Congestive failure, papilledema and a blood pressure of 220/140 mm. Hg caused her first admission to the hospital. Papilledema and macular exudates cleared slowly concomitant with clearing of heart failure, but with ambulation the blood pressure persisted at 220/140 mm. Hg. Figure 49 shows the right fundus one year after the first bout of papilledema, with a suggestion of a remaining postpapilledema halo. Six months later she neglected her cardiac regimen, frank congestive failure supervened and papilledema recurred simultaneously although blood pressures were unchanged. Her failure again responded to treatment and papilledema cleared. A receding phase of this bout of retinopathy is seen in Figure 50 in which the disc, still "mushroomed," is surrounded by a broad dark halo and peripapillary exudates. Four months later the disc had resumed normal size but residual exudates remained. (Fig. 51.)

At this time vigorous therapy with ganglion blocking agents reduced the blood pressure level to a range of 140/110 to 170/120 mm. Hg, yet two months later frank congestive failure and papilledema recurred. Figure 52 shows the nerve head swelling at its inception. Compensation again was restored and papilledema cleared slowly, leaving only scattered hard exudates six months later. (Fig. 53.) Although the administration of hypotensive drugs was continued, no depressor effects could be realized and diastolic levels of 140 mm. Hg have persisted. A year and a half after her last bout of failure and papilledema only residual exudates remain. (Fig. 54.)

HYPERTENSIVE ENCEPHALOPATHY WITH RETINOPATHY

Most observers include hypertensive encephalopathy in the accelerated phase of hypertension, regardless of etiology. Grade 3 or 4 retinopathy is an almost constant companion to the headache, vomiting, convulsions, coma and rise in blood pressure which characterize the syndrome clinically. Since certain of these manifestations are rapidly transient, the fate of the associated retinal changes assumes added importance in clinical follow-up.

Figures 55 and 56 depict progressive worsening of the right fundus of a thirty-three year old white man with accelerated essential hypertension. Headache, blurred vision, vomiting, disorientation and papilledema (Fig. 55) were present. Spinal fluid pressure was 350 mm. of water, and the spinal fluid protein was 120 mg. per cent. Over a two-week period retinopathy increased (Fig. 56), drug and diet therapy were ineffective, and he died in encephalopathy without uremia developing.



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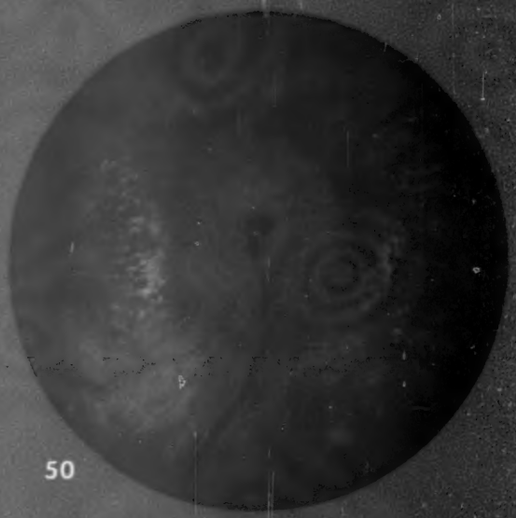

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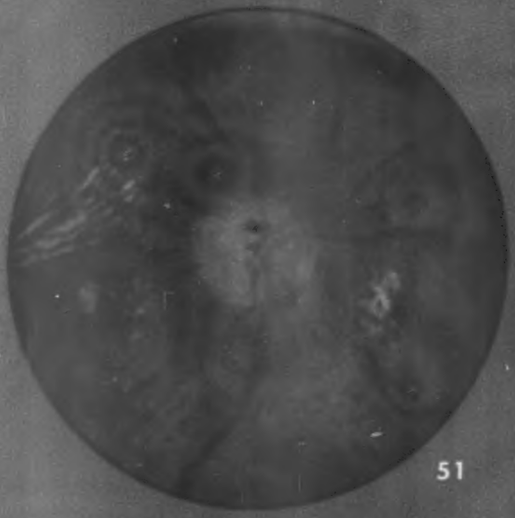

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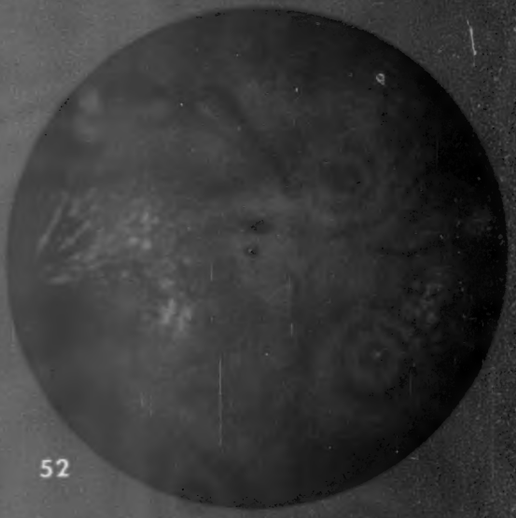

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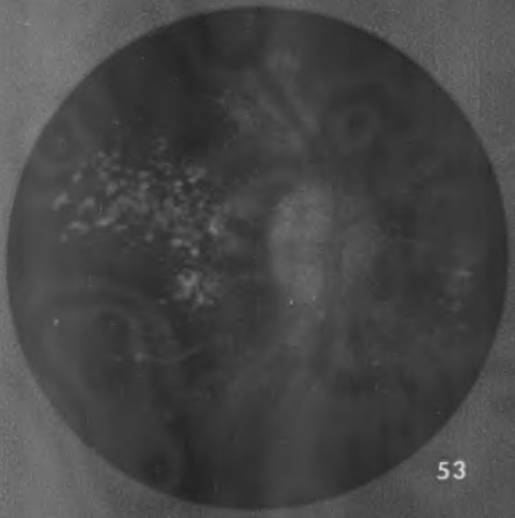

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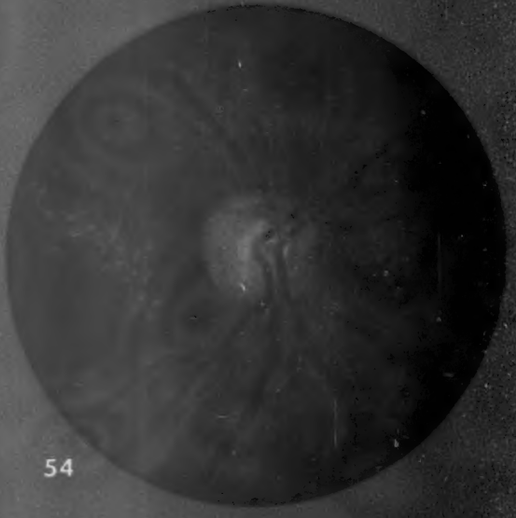

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Fulminant encephalopathy with headache, convulsions, coma and papilledema caused the admission of the thirty-seven year old white man whose right fundus is first pictured in Figure 57. Persistent albuminuria and hematuria were present but uremia had not yet appeared. Spinal fluid pressure was 160 mm. of water and spinal fluid protein 112 mg. per cent. Encephalopathy cleared promptly but receding papilledema was still present two months later (Fig. 58), and he died in uremia shortly thereafter. In Figure 58 the migration of edema residues toward the macula is well shown.

Early uremia was already present at admission of the forty year old white man whose left fundus is shown in Figures 59 and 60. Six months previously sympathectomy had failed to reduce blood pressure, and he had suffered a cerebral accident. Severe abdominal pain appeared as encephalopathy abated. The fundus changed but little from admission (Fig. 59) to one month later (Fig. 60), although uremia had become profound. Autopsy revealed widespread periarteritis nodosa. Again the tendency for edema residues to migrate toward the macula can be seen. (Fig. 60.)

It must be recalled that the preceding three patients died rather quickly, either from encephalopathy or from renal insufficiency. The apparent irreversibility of retinopathy which their examples suggest will not be borne out by those which follow. The fundi often may be observed to clear quite dramatically but as will be seen this retinal improvement may be entirely independent of the clinical course of the underlying disease.

Figures 61, 62 and 63 illustrate rapid clearing of grade 4 hypertensive retinopathy in seven weeks without moderation of blood pressure level. The forty-six year old Negro woman whose left fundus is pictured was hospitalized because of unconsciousness, blindness, left-sided paresthesias, headache and vomiting associated with increased intracranial pressure. Hypertension remained fixed although cerebral symptoms abated promptly.

Several days after admission large fresh hemorrhages were present over the lower portion of the disc and mobilization of edema residues already had begun. (Fig. 61.) Two weeks later (Fig. 62) papilledema largely had dissipated although fresh hemorrhages had appeared along the superior temporal vessels. Retinopathy had disappeared by seven weeks (Fig. 63) and she was discharged asymptomatic. A similar episode one year later culminated in uremia and death.

Chronic pyelonephritis and accelerated hypertension presenting with encephalopathy and gross hematuria caused the admission of the thirty-seven year old Negro whose right fundus is shown in Figures 64, 65 and 66. The blood pressure level remained fixed throughout and uremia was progressive. Overfilled veins and marked papilledema are present in Figure 64 but retinal clearing is apparent one month later. (Fig. 65.) Hypertension persisted, and uremia advanced steadily but the fundi continued to improve over the next month. (Fig. 66.) He died in uremia three weeks later without recurrence of papilledema.

Moderation of blood pressure was achieved with combination drug therapy in the forty-five year old white man whose right fundus is shown in Figures 67 and 68, but his encephalopathy and congestive failure proved refractory to continued treatment. Although papilledema receded somewhat between admission (Fig. 67) and two-month follow-up (Fig. 68), the clinical course was progressively downhill. Death occurred following massive hemorrhage from an unsuspected duodenal ulcer, possibly a complication of drug therapy.

Figures 69 and 70 picture the clearing of hemorrhage and exudate in the left fundus of a forty year old hypertensive Negro woman with chronic pyelonephritis. Ganglion blocking agents did not reduce the diastolic blood pressure below 150 mm. Hg, although encephalopathy symptoms remitted and congestive failure was controlled. The grade 3 changes present in the first photograph (Fig. 69) had disappeared completely ten weeks later. (Fig. 70.)

A forty-six year old Negro woman with chronic renal disease showed hemorrhages, exudates and blurred disc margins during her admission for hypertensive encephalopathy. The right fundus is seen in Figure 71. The blood pressure level was not lowered by the administration of drugs. Transient hemorrhages and exudates which recurred during the follow-up period had cleared at the end of five months (Fig. 72) although hypertensive levels remained unchanged.

The differential diagnostic help sometimes contributed by accurate retinal evaluation is illustrated by Figure 73. The thirty-five year old Negro woman whose left fundus is pictured was admitted with a diagnosis of hypertensive encephalopathy presenting with severe headache, nausea and vomiting. Both fundi showed papilledema but the single hemorrhage present (Fig. 73) was dark-centered and surrounded by a red halo, resembling the subhyaloid retinal hemorrhages seen with subarachnoid bleeding. (Fig. 20.) Spinal fluid findings and subsequent clinical developments confirmed subarachnoid hemorrhage as the correct diagnosis. Ganglion blocking agents reduced blood pressure and the subhyaloid hemorrhage resorbed in six weeks (Fig. 74) although residual papilledema persisted longer.

In hypertensive encephalopathy grade 3 and grade 4 retinopathy occasionally may be entirely unilateral. More often, the changes in one eye will be more severe and will clear more slowly than in the other, as shown in Figures 75–78. This forty-three year old white man, admitted with encephalopathy and congestive failure, had grade 4 fundi bilaterally but the temporal disc margin remained visible on the right (Fig. 75), whereas papilledema obviously was more pronounced on the left. (Fig. 77.) The blood pressure fell dramatically with control of the heart failure before depressor drugs could be administered. Two months later the right fundus has cleared completely (Fig. 76) while the left still shows residual exudates. (Fig. 78.)

RETINAL VASCULAR CHANGES IN CHRONIC RENAL DISEASE

Earlier photographs have demonstrated certain exudative manifestations which may occur with acute and chronic renal disease, classic examples of which are the so-called "albuminuric retinitis" and the macular star figure. It has previously been suggested that the slower resorption of exudates in patients with chronic renal impairment reflects a functional inadequacy of the retinal vessels, possibly the result of hypertensive damage. The following examples lend photographic support for such a concept by illustrating the apparent disappearance of small calibre retinal vessels (both arterial and venous) in patients with prolonged renal insufficiency.

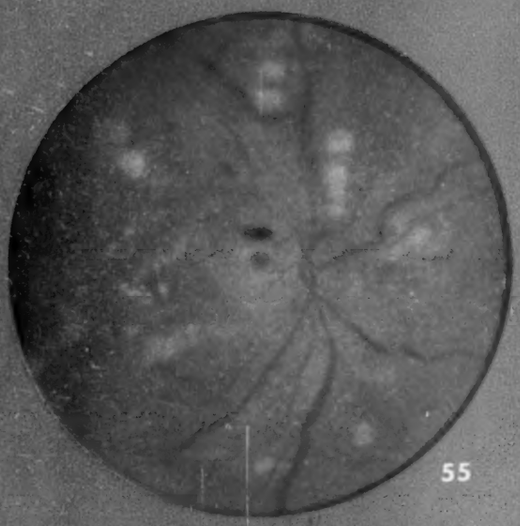
The thirty-five year old Negro whose left fundus is shown in Figures 79 and 80 had chronic glomerulonephritis proved by renal biopsy. At his first admission hemorrhages and exudates were present; details of the vessels and their branches are clearly seen in Fig. 79. One year later (Fig. 80) multiple white punctate exudates obscure the fundus and the smaller vessels are no longer visible.

A two-year interval elapsed between the taking of Figures 81 and 82, the left fundus of a forty year old Negro woman with chronic glomerulonephritis confirmed by renal biopsy. In Figure 81 the smaller branches of both the superior and inferior temporal vessels are well seen but these disappeared from view two years later. (Fig. 82.) A recent bout of papilledema, still incompletely cleared, accounts for the larger size of the disc in Figure 82.

A forty-six year old Negro woman with chronic pyelonephritis was the subject for the right fundus views in Figures 83 and 84. In Figure 83 she had recently recovered from encephalopathy and the smaller arterial and venous branches show clearly. Just prior to her terminal episode a year later (Fig. 84) even the larger vessels are obscured a short distance from the disc.

Diabetes, hypertension and chronic renal insufficiency were present in the thirty-three year old Negro woman whose right fundus is pictured in Figures 85 and 86. Renal biopsy had confirmed the diagnosis as intercapillary glomerulosclerosis prior to admission with encephalopathy and congestive failure. Figure 85 shows a few capillary aneurysms, scattered exudates and overfilled veins, although papilledema was not present. Ten months later (Fig. 86) many of the smaller vessels have disappeared or their previous courses are indicated by bloodless channels. New growth of tiny vessels over the disc (proliferating retinitis) is apparent.

The sudden appearance and rapid clearing of retinopathy associated with an unusual form of renal disease is illustrated by Figures 86 and 88. In the previously well thirty-two year old Negro woman whose left fundus is pictured anuria and hypertension developed following abruptio pla-



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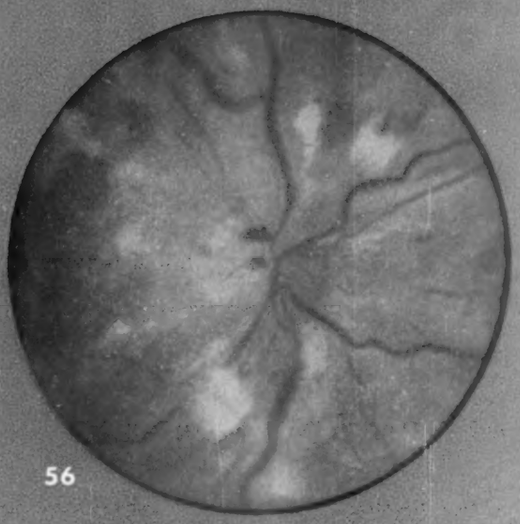

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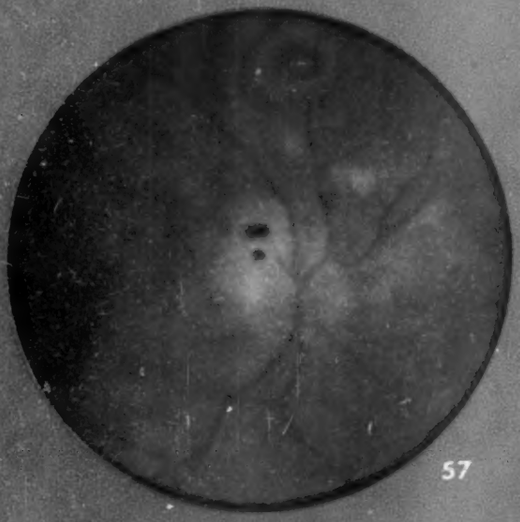

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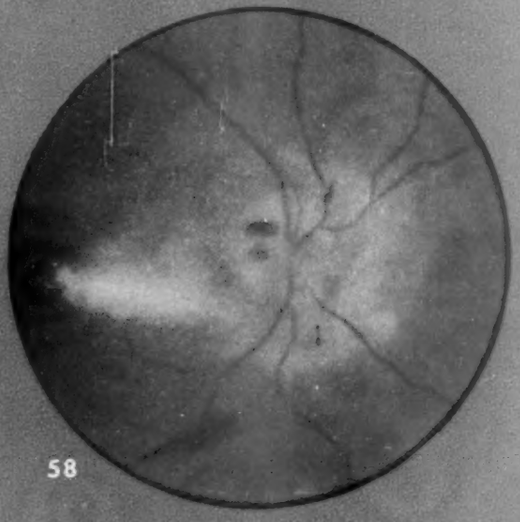

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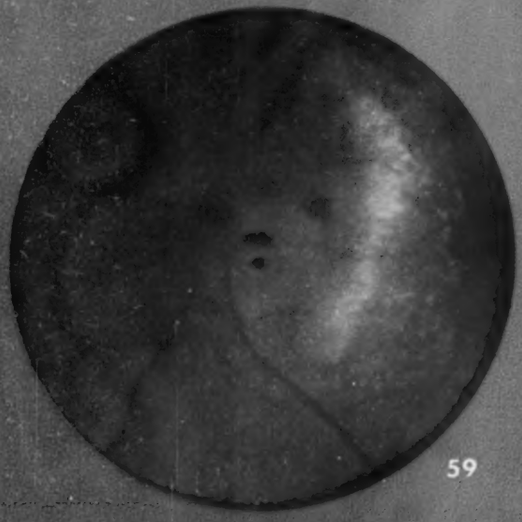

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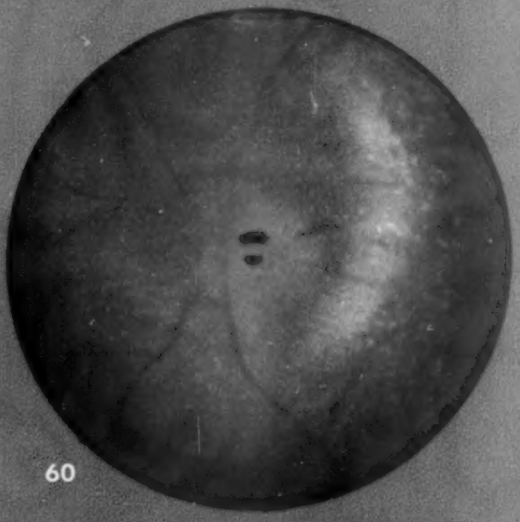

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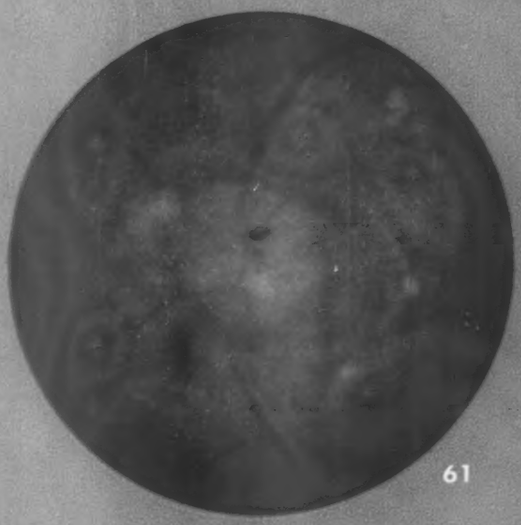

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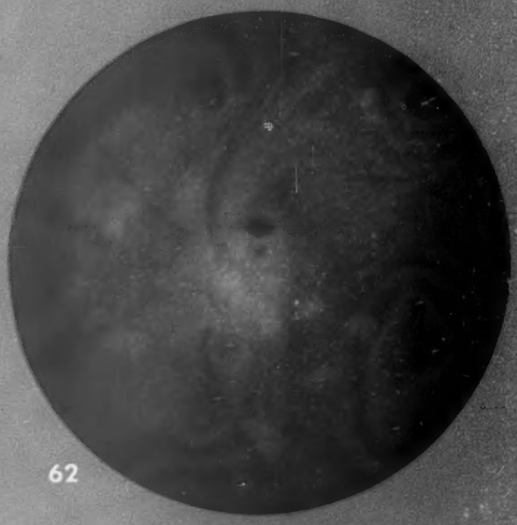

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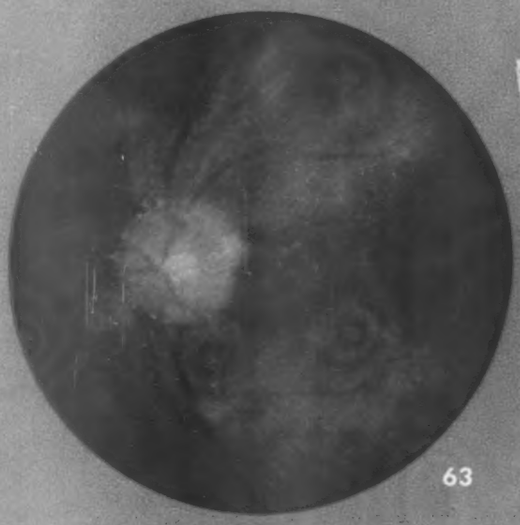

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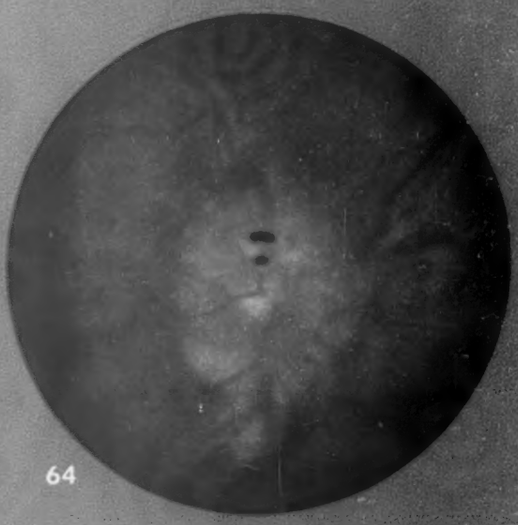

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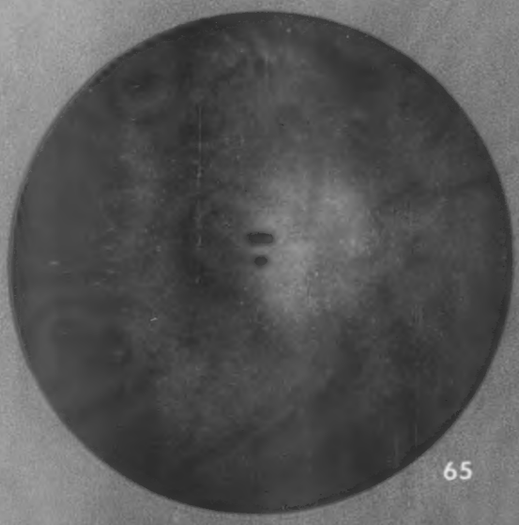

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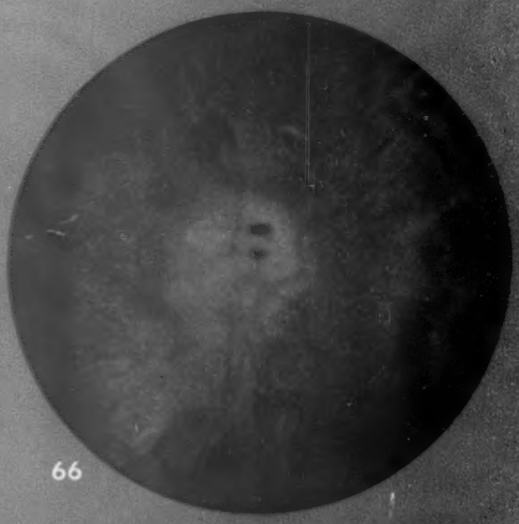

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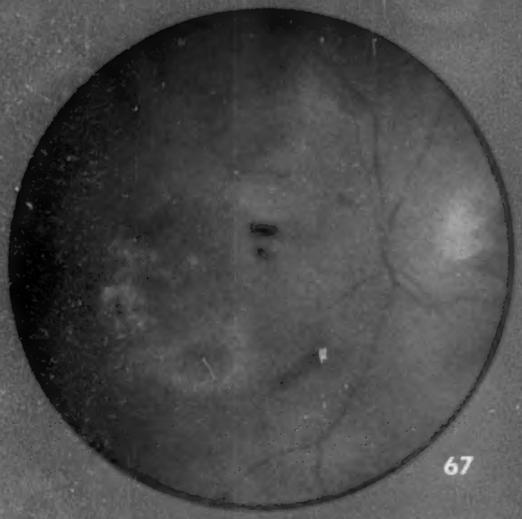

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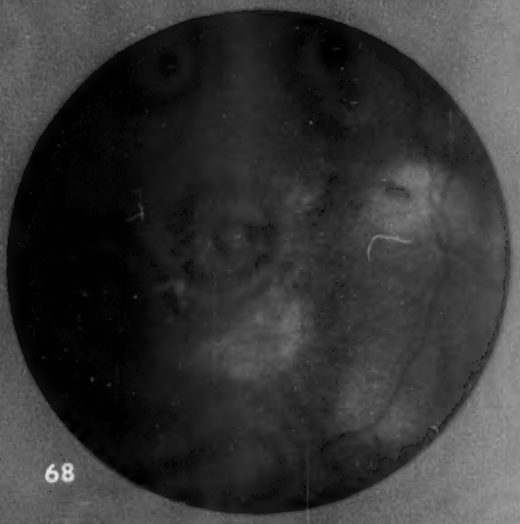

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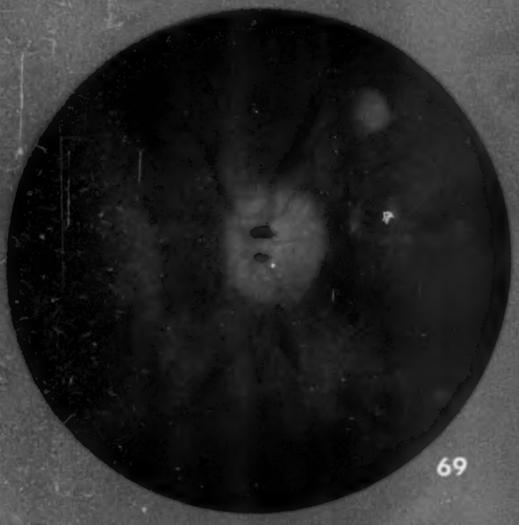

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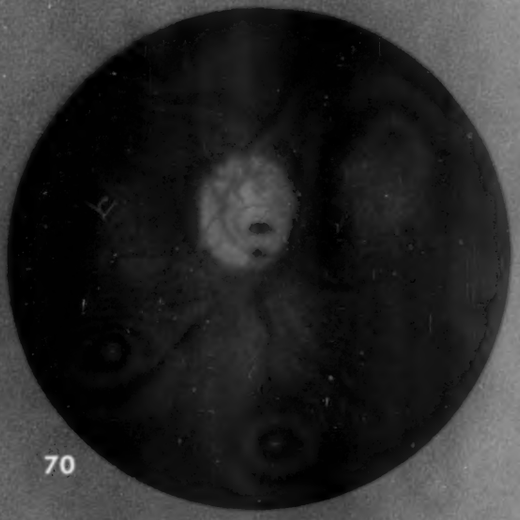

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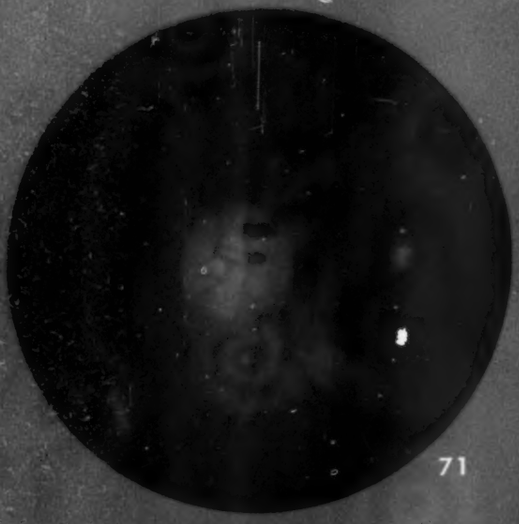

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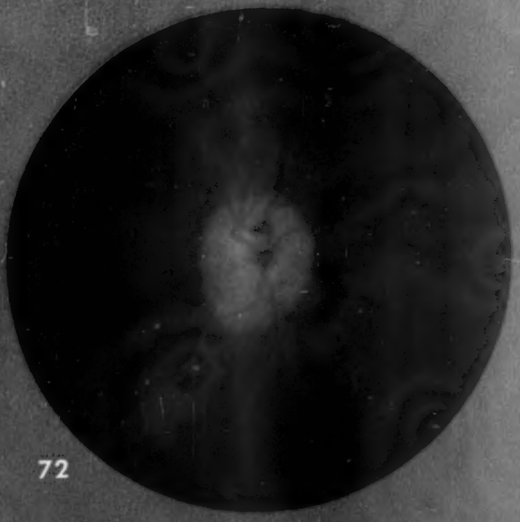

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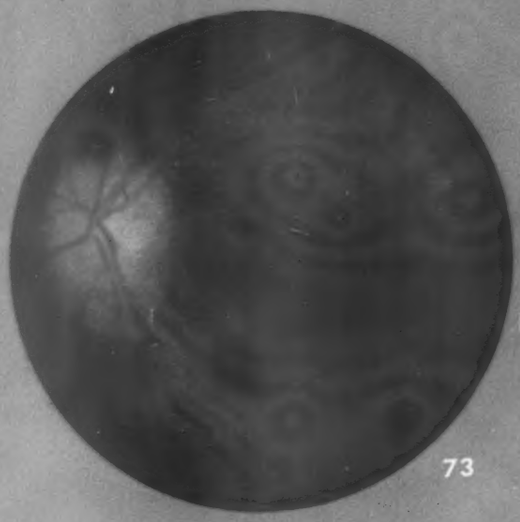

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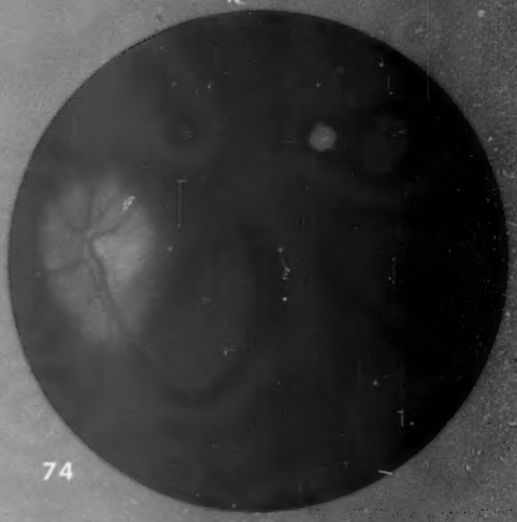

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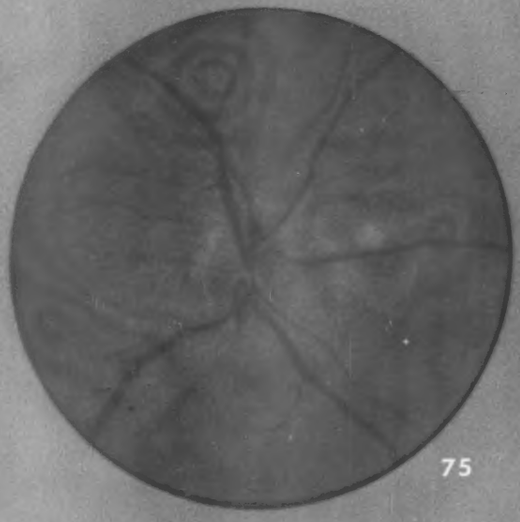

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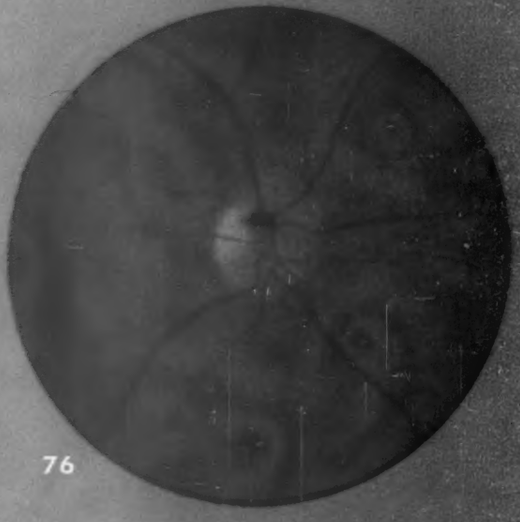

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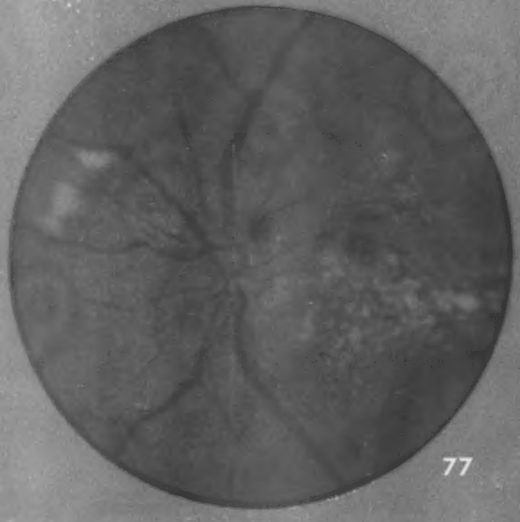

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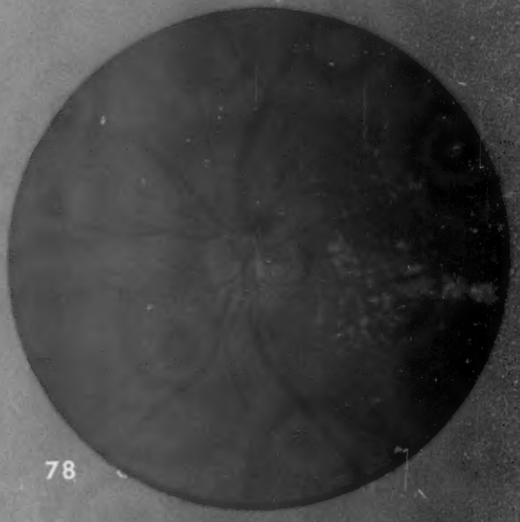

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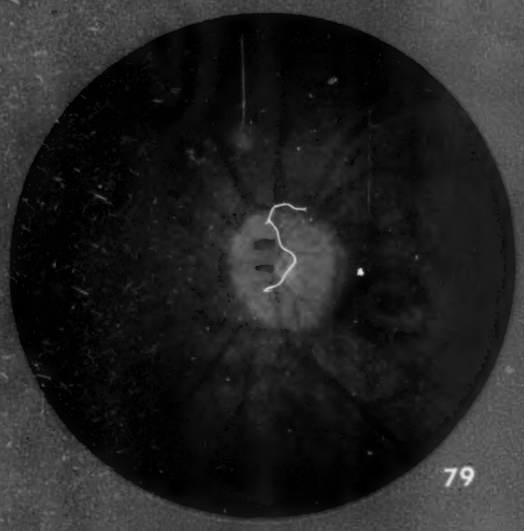

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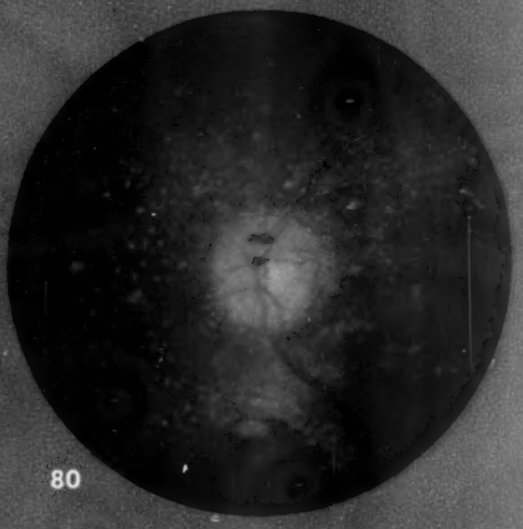

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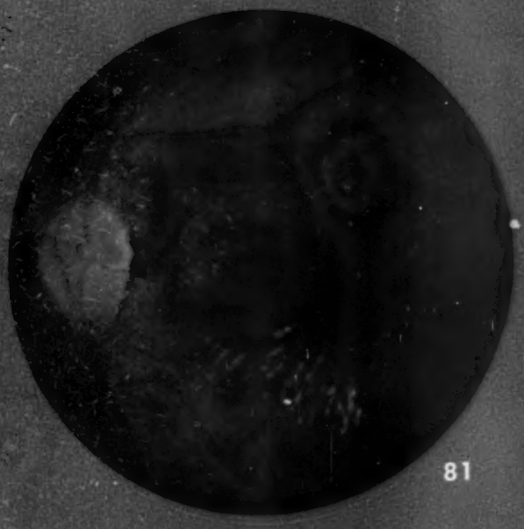

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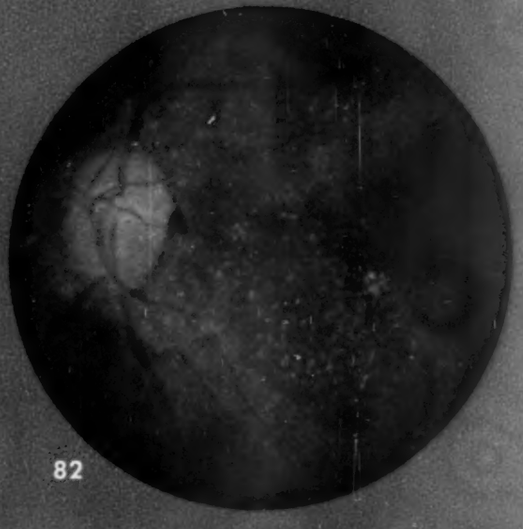

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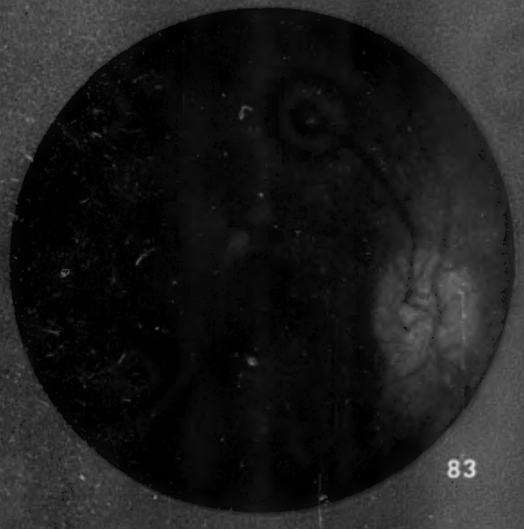

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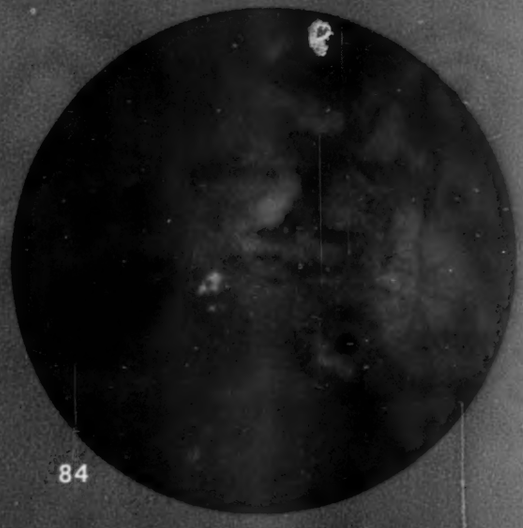

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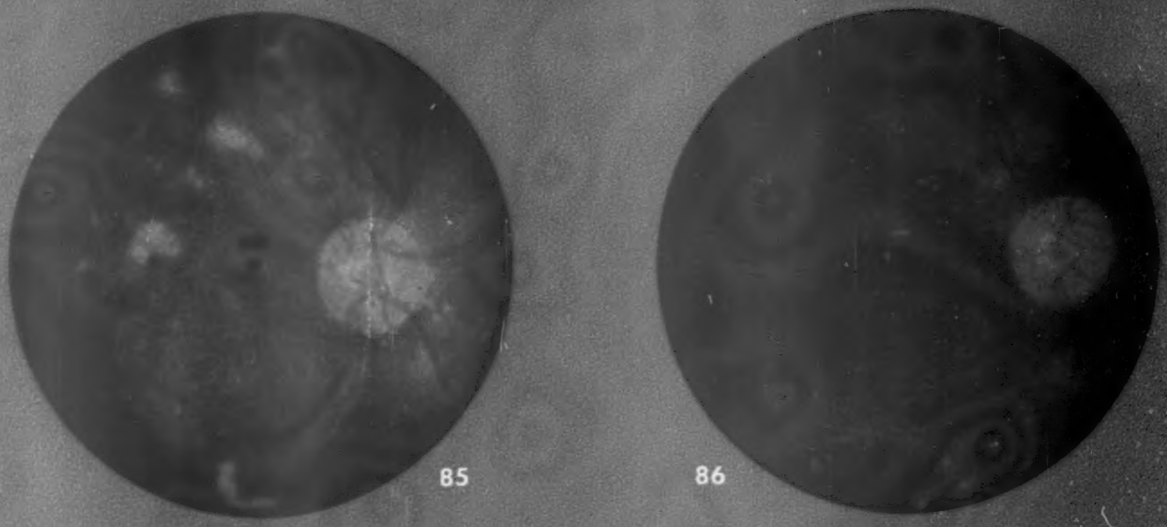

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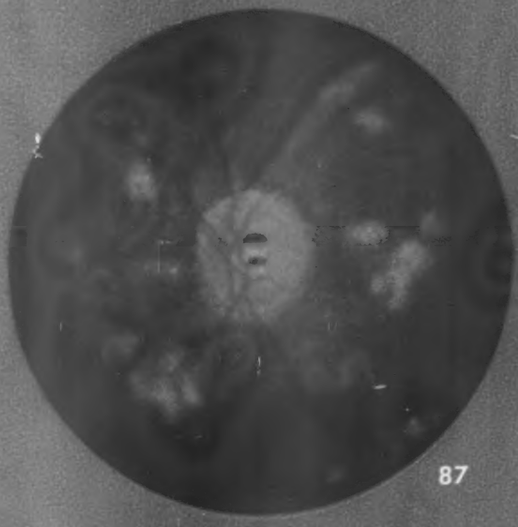

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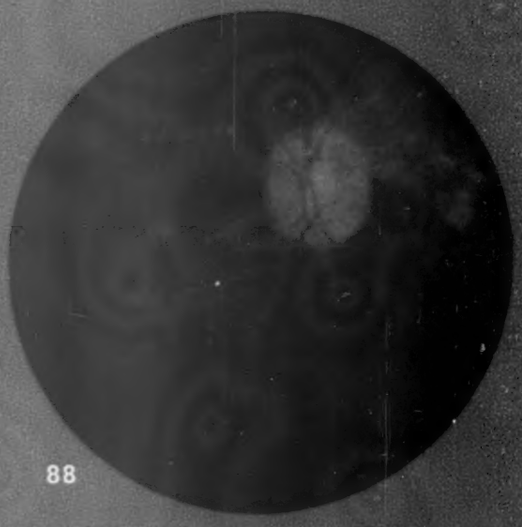

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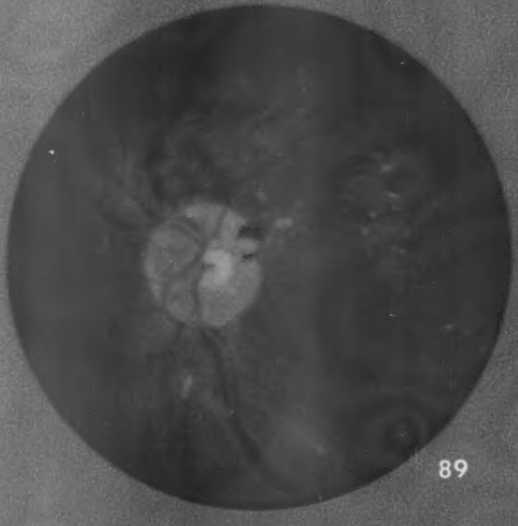

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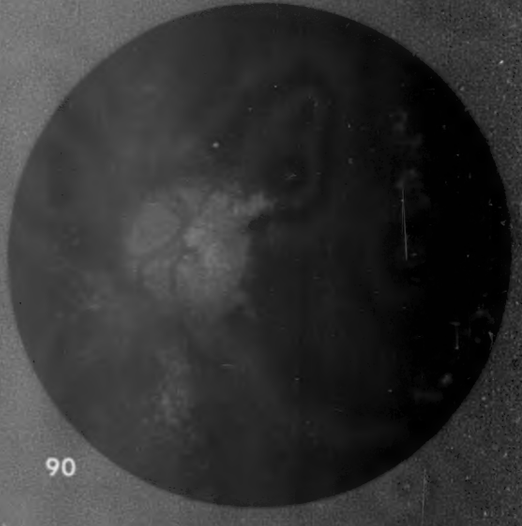

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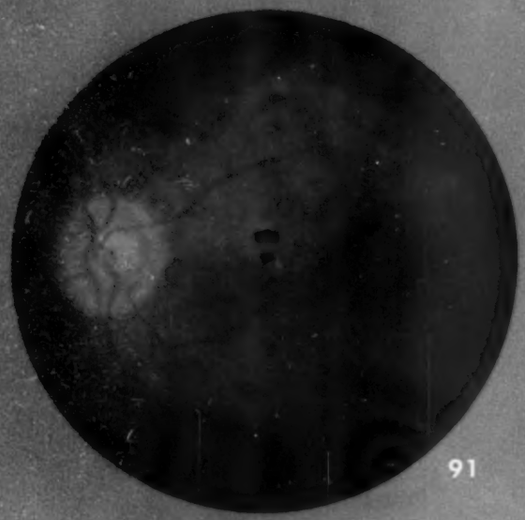

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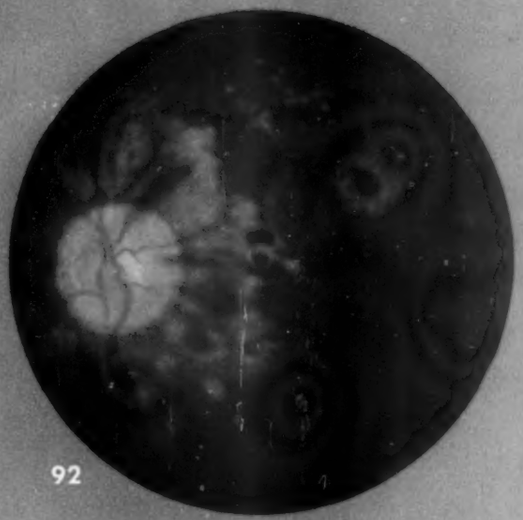

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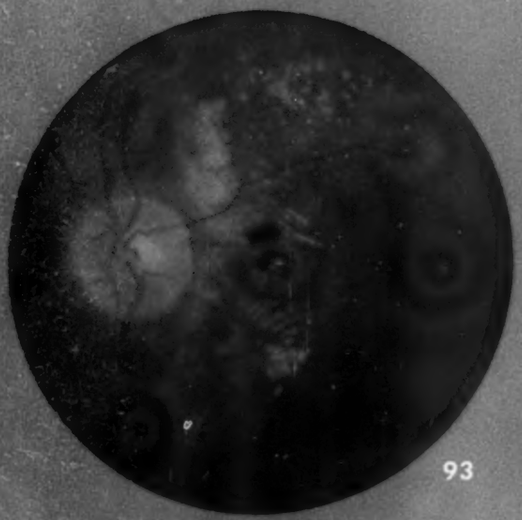

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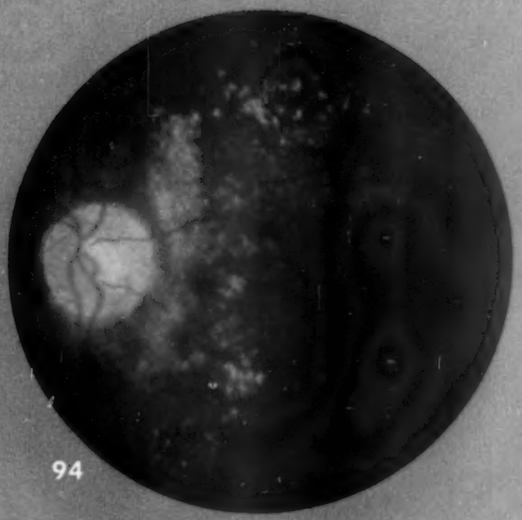

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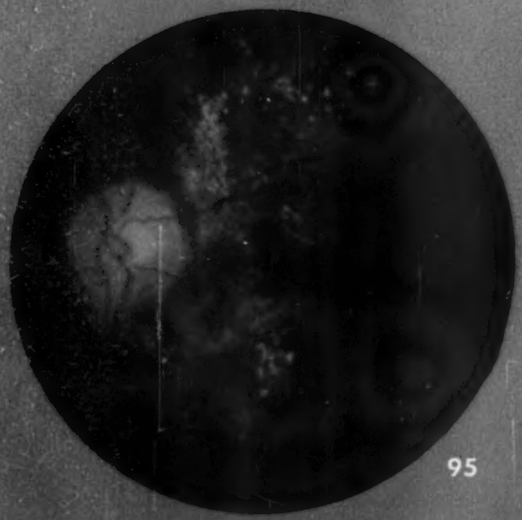

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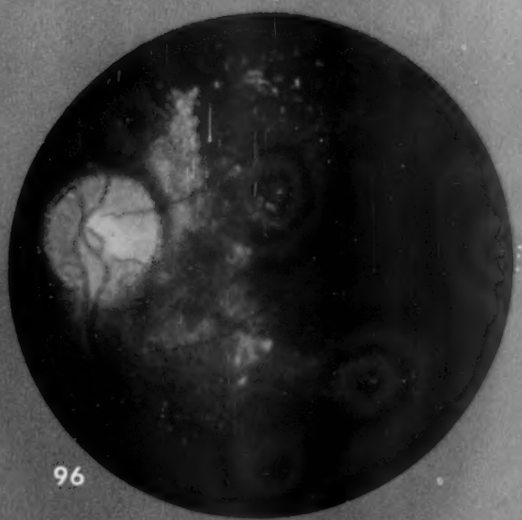

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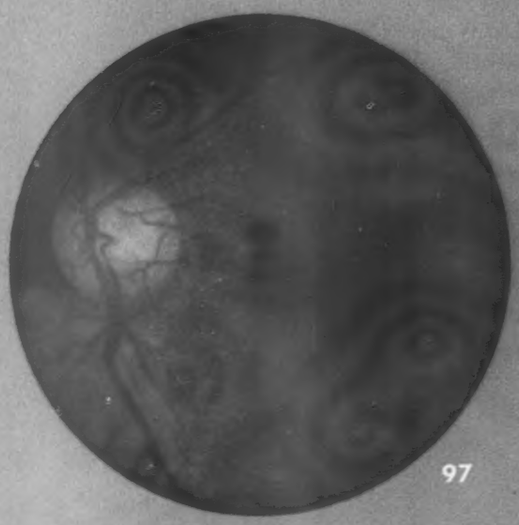

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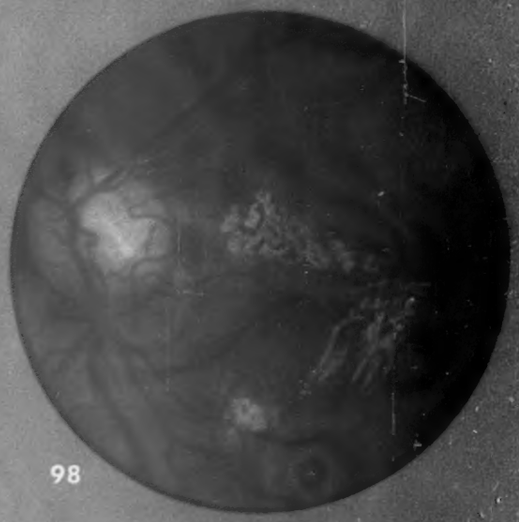

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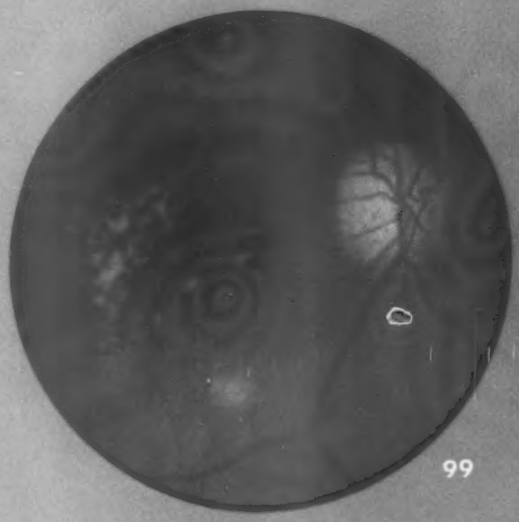

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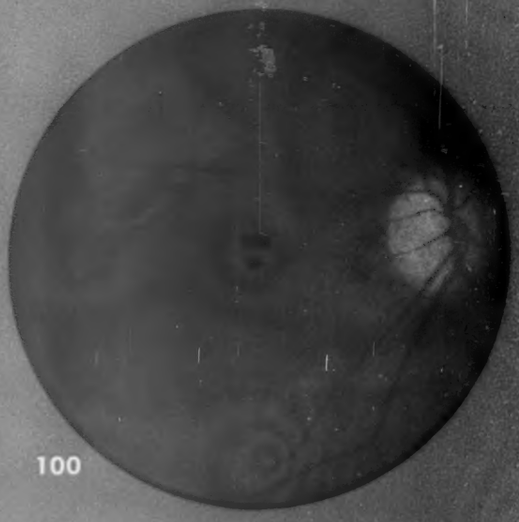

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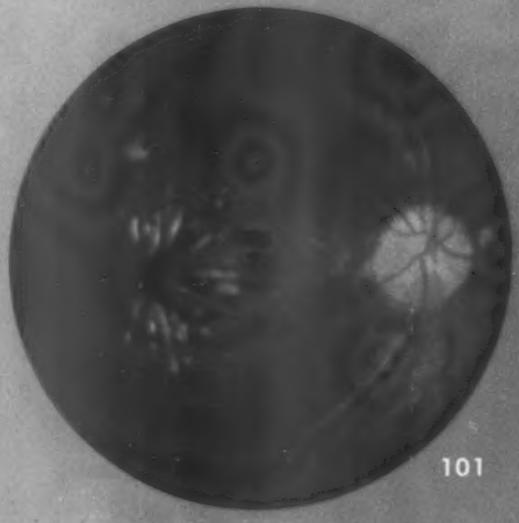

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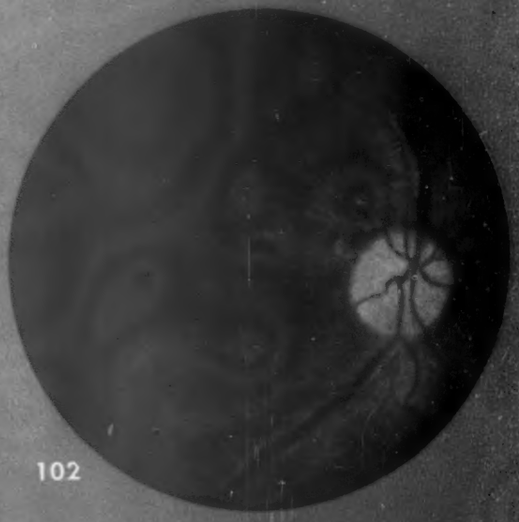

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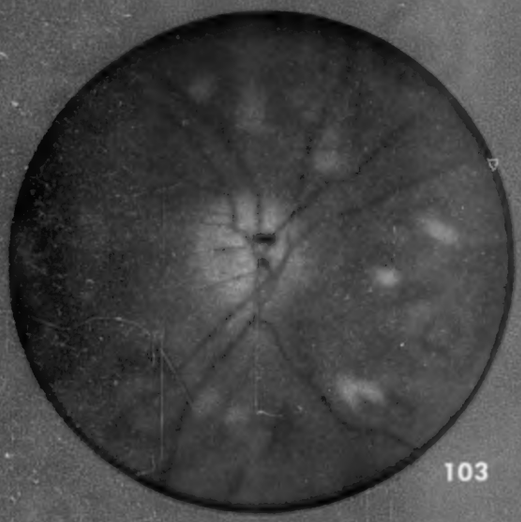

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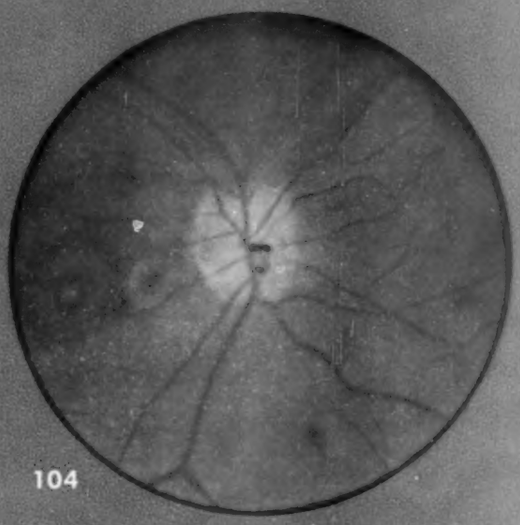

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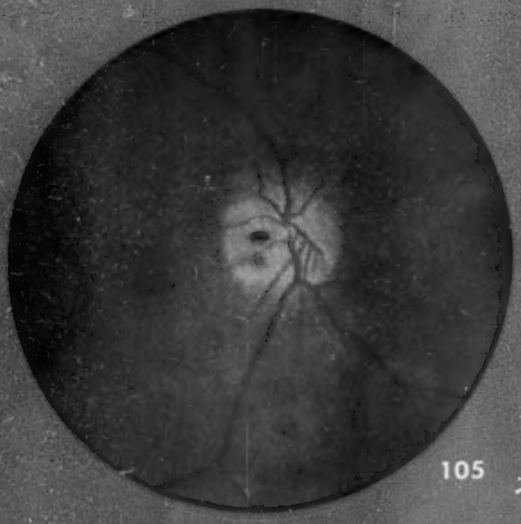

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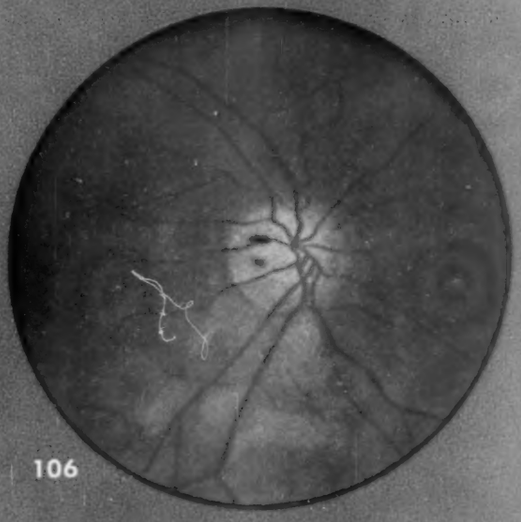

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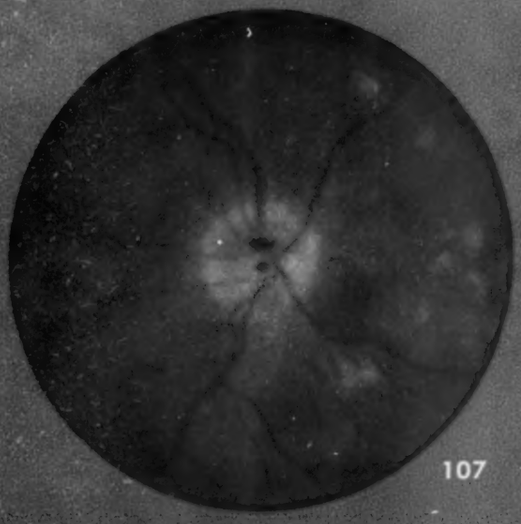

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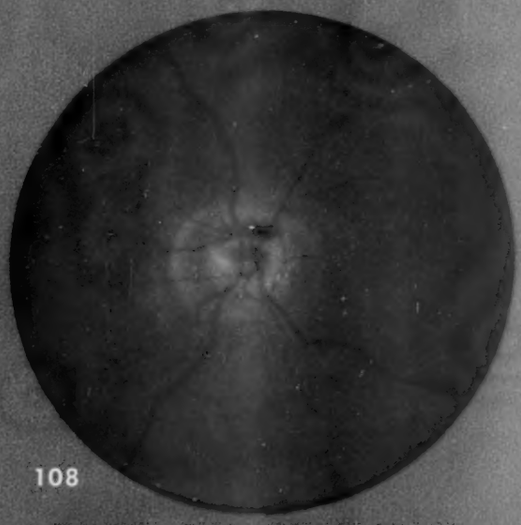

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centa complicating her second pregnancy. The diagnosis of cortical necrosis of the kidney was confirmed by needle biopsy. Figure 87 shows a profusion of fluffy white and yellow exudates which appeared acutely. There was no evidence of previous retinal vascular disease in this patient, and in just one month (Fig. 88) most of the exudates had cleared although hypertension persisted.

Severe refractory anemia apparently secondary to the uremia of chronic pyelonephritis complicated the course of the forty-seven year old Negro woman whose left fundus is shown in Figures 89 and 90. In Figure 89, just after the onset of uremia, the smaller retinal vessels still can be seen reasonably well, but two and a half years later (Fig. 90) they are no longer visible. These fundi never cleared of punctate exudates during a four-year follow-up.

RETINOPATHY AND THE INFLUENCE OF ANTIHYPERTENSIVE AGENTS

Previous photographs have emphasized that the first appearance of advanced retinopathy (grade 3 and grade 4) correlates reasonably well with other clinical evidence that hypertension has entered an accelerated phase. On the other hand, the clearing of such advanced retinal changes could not be correlated consistently either with blood pressure level or with the further course of the disease.

Since antihypertensive agents are generally acknowledged to have improved the prognosis of accelerated hypertension, the following clinical examples are included to illustrate the course of certain retinal changes with relation to the vigorous exhibition of depressor drugs.

Figures 91–96 picture serial changes seen in the left fundus of a thirty-five year old Negro woman, known to have had hypertension for three years. She presented with a blood pressure of 210/150 mm. Hg and a single linear hemorrhage adjacent to the left disc margin. (Fig. 91.) Laboratory studies were within normal limits and since symptoms were minimal she refused treatment initially. Three months later papilledema, multiple hemorrhages and exudates appeared (Fig. 92) and she was admitted with encephalopathy. Administration of ganglion blocking agents reduced blood pressures taken with the patient sitting and standing to the normal range although readings with the patient supine remained elevated. Encephalopathy cleared promptly and papilledema and retinal hemorrhages disappeared in three weeks (Fig. 93) although edema residues and exudates persisted. Blood pressure levels were controlled reasonably well by combination drug therapy but further resolution and absorption of exudates remained almost stationary, as shown at monthly intervals of follow-up. (Figs. 94, 95 and 96.) Although the administration of drugs was continued, uremia appeared and she died two months later.

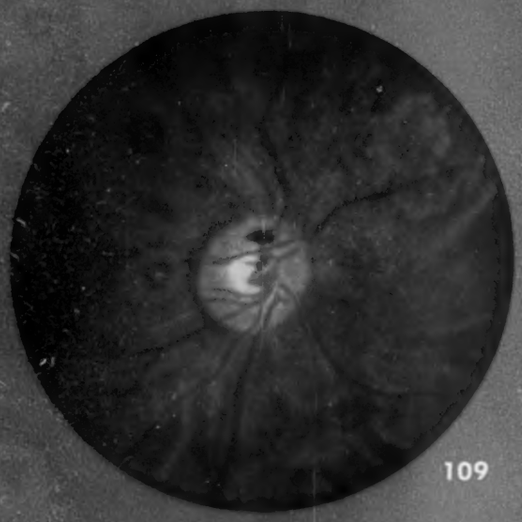
Antihypertensive agents were administered in large dosage with disappointing effects on blood pressure in the three patients whose fundi are illustrated in Figures 97–102. During treatment new macular exudates appeared in one patient while similar exudation was observed to resorb in the other two.

Combined drug therapy failed to reduce the blood pressure of the forty-two year old white woman whose left fundus is pictured in Figures 97 and 98, but hemorrhage and exudate previously present had cleared by the time of the first photograph. (Fig. 97.) The administration of drugs was continued without apparent effect on blood pressure and the fundi remained clear until six months later when macular exudates were noted. (Fig. 98.) Signs of renal insufficiency have not appeared, and the exudates have resorbed slowly.

In Figure 99 the right fundus of a twenty-nine year old white woman shows slight papilledema and macular exudates resulting from encephalopathy two months before. Initial profound sensitivity to ganglion blocking agents was lost as encephalopathy cleared and further drug therapy did not affect the blood pressure. Nevertheless retinal clearing continued as indicated by Figure 100, taken eight months later.

A complete macular star figure is seen in the right fundus of a thirty-six year old Negro woman in Figure 101. She had recovered from encephalopathy three months before. Administration of depressor agents in large doses did not affect blood pressure, but one year later (Fig. 102) the macular region is clear.

Progressive retinal improvement as indicated



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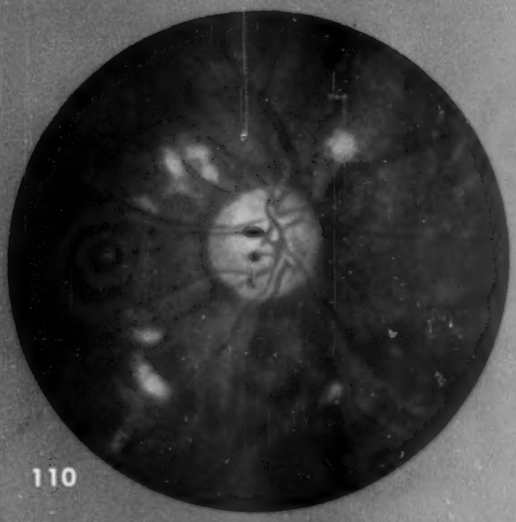

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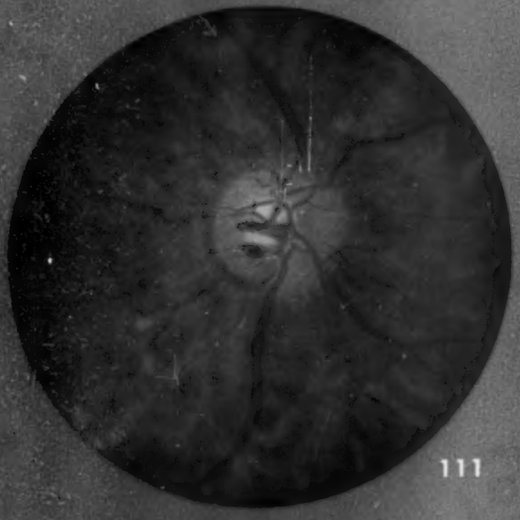

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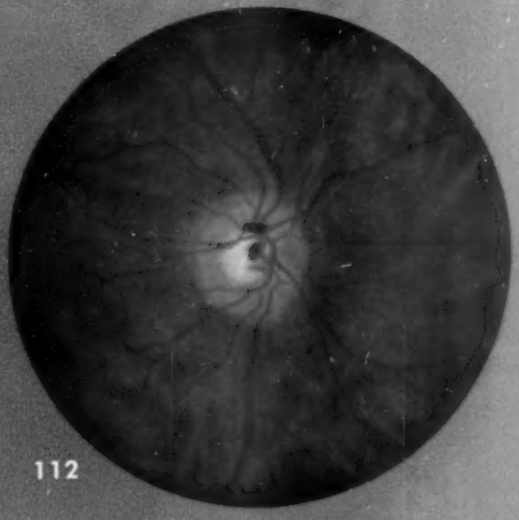

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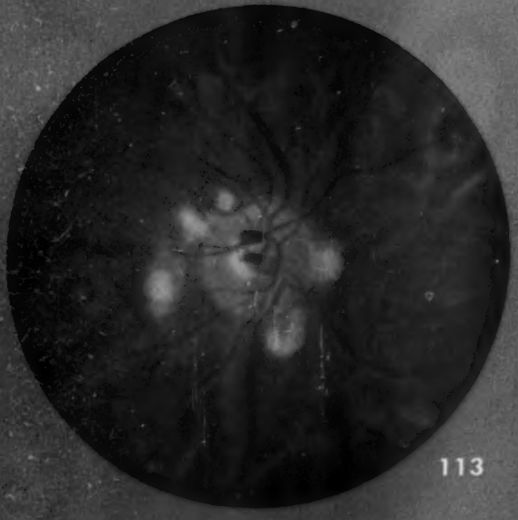

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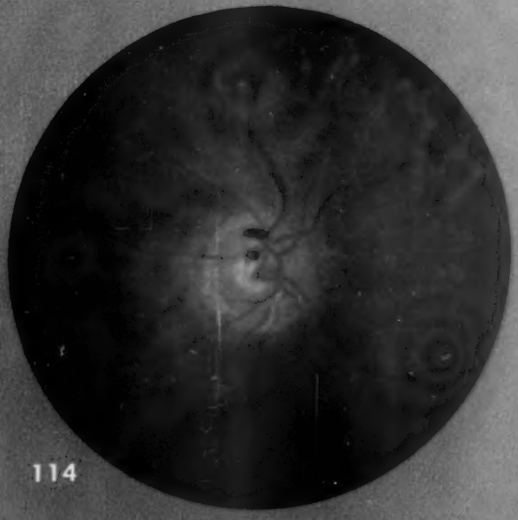

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by serial photographs in the year following fulminating encephalopathy is illustrated in Figures 103–106. Convulsions and coma caused the admission of this twenty-nine year old white woman in whom sympathectomy had failed to affect blood pressure level. Papilledema, hemorrhages and exudates were present as pictured. (Fig. 103.) Although ganglion blocking agents were dramatically depressor during encephalopathy, responsiveness did not continue thereafter. Three months later (Fig. 104) papilledema was subsiding and exudates had resorbed, but fresh hemorrhages were still appearing. Drug therapy was continued without effect on blood pressure, and photographs at six months (Fig. 105) and one year (Fig. 106) show complete retinal clearing. Death occurred two years later following massive intracerebral hemorrhage. The persistent inability to reduce blood pressure was possibly explained by the finding of a renal artery occlusion and shrunken kidney at necropsy.

Bilateral pheochromocytomas were a surprise finding at necropsy of the forty-three year old white man whose left fundus is reproduced in Figures 107 and 108. Coma and convulsions with retinal hemorrhages, exudates and papilledema (Fig. 107) caused his admission. A dramatic fall in blood pressure levels was attributed to administration of antihypertensive drugs but recrudescence of old ulcer symptoms forced their discontinuance and moderate persistent hypertension returned. Five months later the fundus had cleared. (Fig. 108.) Myocardial infarction and congestive failure caused his death the following year.

Figures 109–114 illustrate the changes in the right fundus observed over the course of one year in a forty-three year old Negro. His hypertension had been documented for eleven years in the range of 210/120 mm. Hg and several bouts of congestive failure had occurred. When combination drug therapy including a ganglion blocking agent was instituted the blood pressure was 220/130 mm. Hg and the retinal changes were grade 2. (Fig. 109.) A gratifying blood pressure response occurred and his levels were sustained in the 150/100 mm. Hg range, yet two months later (Fig. 110) hemorrhage and cotton wool exudate appeared abruptly. He continued responsive to drug therapy and within a month (Fig. 111) retinal clearing was apparent. Lowered blood pressure was maintained as before and clearing was complete in four months. (Fig. 112.) However, during the next three months medication became progressively less effective, the blood pressure rose, cotton wool patches appeared in the fundus (Fig. 113) and congestive failure supervened. Although failure became intractable and the blood pressure persisted at 240/150 mm. Hg, the exudative changes had disappeared two months later. (Fig. 114.) Death finally resulted from massive intracerebral hemorrhage.

SUMMARY

Representative color photographs of the ocular fundi are presented to demonstrate anatomic features which figure prominently in determining the characteristic configuration of certain vascular retinopathies. Serial photographs in the same patients have been employed to study the course of hypertensive retinopathy with relation to involvement of the brain, heart and kidneys and to changes in blood pressure level.

In the accelerated phase of hypertension advanced retinopathy may appear and progress in spite of moderation of the blood pressure. Conversely, regression of hemorrhages, exudates and papilledema may occur without lowering of blood pressure level, even in the presence of advancing uremia.

Such observations do not detract from the diagnostic value of the funduscopic examination; rather they emphasize a need for caution in interpreting retinal changes as indicative of the success or failure of antihypertensive therapy.

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Seminar on Connective Tissue

Some Notes on the Composition and Metabolism of Connective Tissue*

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The connective tissue is of mesodermal origin and comprises a group of tissues which undergo varying degrees of differentiation, mainly in response to the mechanical requirements of the developing organism. They are, indeed, the system which "connects" and supports the more highly specialized organs of the body. To the student in the dissecting room they may seem a plain nuisance, something to be removed in exposing nerves, blood vessels and "vital" organs. Later in his career he may find this attitude continued in the autopsy room. Their many mechanical functions are plain enough, providing a framework for the total organism, providing gliding planes for both solid and soft tissues, and in the repair of injury. Less obvious is the role that some, at least, of the connective tissue constituents play in the dynamic equilibrium of the body. Upon the integrity of the connective tissues depends the very life of the organism. The living cells of the microcosm have existence only so long as their immediate environment, the connective tissue, enables the exchange of water, ions and all the new materials for the cell enzyme systems.

We will be concerned here first with the composition of the connective tissues mainly in the biochemical sense, and later with the metabolism of some of its constituents. But, before descending to molecular levels, a list of the components defined at the histological level would seem desirable. Taking loose connective tissue, areolar tissue, as an example, microscopic examination reveals a system of cells and fibres embedded in a substratum of semifluid gelatinous substance, the so-called ground substance. Of the various cells described two are specially common, the fibroblasts and the histiocytes. Also to be found in connective tissue are the mesenchyme cells, mast cells, plasma cells, fat cells and pigment cells. In considering the composition of connective tissue probably the most important of these cells are the fibroblasts and mesenchyme cells; the former because there is good evidence that they, and their variants, e.g., the chondroblasts and osteoblasts, control the formation of the extracellular constituents of connective tissue; the latter, the mesenchyme cells, because they appear to be primitive cells capable of differentiation into more specialized elements.

Although relatively acellular when compared with an organ such as the liver, the cells may often represent a considerable bulk of the connective tissue. For example, it is estimated that twenty to thirty per cent of such a tissue as cartilage consists of cells [*11*]. This is a fact worth bearing in mind when one attempts to evaluate the results of any biochemical studies on connective tissue. Outside the cells lie the fibrous elements of connective tissue and the ground substance. The fibrous elements are recognizable under the microscope as collagen, elastin and reticulin, on the basis of their appearance and staining reactions. The ground substance can be demonstrated as an amorphous matrix by special staining reactions but there is no clue, at this level of examination, to the complex nature of the inter-cell system of mucopolysaccharides, proteins and solute. The relative amounts of fibres, cells and ground substance show considerable variations in the different kinds of connective tissue. As would be expected there is a high collagen content where structural strength is called for, as in tendons of which collagen forms some 25 to 35 per cent of

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TABLE 1

COMPOSITION OF SOME FIBROUS PROTEINS IN COMPARISON WITH OVALBUMIN

Data	Ovalbumin (Tristram [44])	Collagen (Bowes [6])	Reticulin (Windrum [45])	Acid-Soluble Collagen (Bowes [6])	Elastin (Bear [3])
Glycine	3.6	26.7	24.6	27.5	35.0
Alanine	6.7	8.7	7.7	8.8	18.8
Valine	6.1	1.6	2.1	1.5	13.0
Leucine } Isoleucine }	12.4	3.2	4.3	2.8	9.0
Phenylalanine	4.1	1.1	1.4	1.0	2.7
Tyrosine	1.8	0.4	0.2	0.2	....
Serine	6.9	3.1	3.4	3.2	0.7
Threonine	3.0	1.4	1.7	1.5	0.8
Cystine	1.4	.....	.....	.....	0.2
Methionine	3.5	0.5	0.6	0.4	1.2
Proline	2.6	9.4	7.7	9.0	11.5
Hydroxyproline	0.0	7.4	8.6	8.2	1.3
Arginine	11.7	14.2	14.4	15.2	2.0
Histidine	4.1	1.0	1.3	0.05	0.1
Hydroxylysine	0.0	1.1	1.9	1.3	....
Lysine	7.7	4.1	3.4	3.9	0.5
Aspartic acid	6.2	3.9	4.2	3.6	0.3
Glutamic acid	10.0	5.7	6.1	5.9	1.6
Amide N	7.8	3.5	3.4	2.9	0.3
Total protein N	15.8	18.6	.....	17.7	16.9
Carbohydrate	....	0.26	4–5	0.02 }	
Hexosamine	....	0.05	0.16	0.01 }	0–12
Lipid	....	.....	10–12	..... }	

Amino acids are in gm. N per 100 gm. protein N.
Amounts in the bottom four lines are percentages dry weight of original material.

the total wet weight. By contrast, the vitreous humour has only about 0.05 per cent collagen in terms of total wet weight [24].

Any consideration of living tissue must necessarily be fragmentary, since such knowledge as we have of it is a synthesis of observations which often entail radical changes in the initial, complete structure. We have very little idea of how the total structure works. Therefore the following subdivisions under which the connective tissue components will be discussed are purely arbitrary. It can only be hoped that they will serve as pegs on which to hang some of the information available.

THE PROTEINS OF CONNECTIVE TISSUE

Fibrous Proteins. The Collagens: The fibrous proteins form the key structural element universally distributed throughout connective tissue. They constitute a family of structural proteins, old in the evolutionary sense, being found in invertebrates as well as in vertebrates. Despite many differences in particulars they have a basic similarity of structure at the molecular level. They all give a distinctive kind of large-angle x-ray fibre diagram, with a meridional reflection spacing of 2.86 Å. This fundamental molecular characteristic is consistent with the concept of a collagen unit consisting of a triad of helices, derived from a polyglycine-polyproline structure, twisted round one another in a kind of coiled coil. Many of the collagens also give the small-angle x-ray diagram, corresponding in period (about 640 Å) to the bands seen, so characteristically, in the electron microscope. Chemically they are distinguished by an unusual pattern of amino acid distribution. (Table 1.)

This pattern is remarkable for the low amounts of tyrosine and sulphur-containing amino acids, and for the high content of glycine, proline and hydroxyproline. These last three amino acids account for some two-thirds of the residues. Only the collagens contain large amounts of hydroxyproline. Indeed it occurs elsewhere only in small amounts in elastin and enamel keratin. Some hydroxylysine is found in collagen too, and in collagen only. Within the family of collagens there are some variations in the relative amounts of the component amino acids. In the collagens of higher animals the variations appear to be small. For example, reticulin has more hydroxyproline and less proline than insoluble collagen, whereas acid-soluble collagen also has more hydroxyproline than insoluble collagen but the amounts of proline are similar. In lower animals collagens, defined by x-ray analysis and electron optics, are found which contain only small amounts of proline and hydroxyproline. However, from this point on we will be concerned solely with the mammalian collagens.

In the body collagen is organized into a system of *fibres* with a range of diameters of the order of 1 to 200 microns. These are formed of submicroscopic *fibrils* whose diameters may range from a few hundred to several thousand Ångström units. Smaller still are the subfibrillar units, *filaments* and *protofibrils*. To this last order of dimension belongs the *tropocollagen*, characterized by Schmitt, Gross and Highberger [*37,38*], which is believed to be the fundamental building block of the fibril. The particle is a rigid rod of the order of 2,000 to 3,000 Å long and less than 50 Å wide. Hall [*15*] has been able to visualize the tropocollagen particles directly by electron optics and has arrived at a mean length of 2,200 Å and a width of about 15 Å. Successive polymerizations and aggregations from such units result in the characteristic banded fibrils seen by electron optics, and ultimately in the more familiar collagen fibres. It is known that the polymerization can occur *in vitro* in the absence of identifiable acid mucopolysaccharide and non-collagenous protein. But the most highly purified collagen fractions analysed seem to contain a small quantity of sugar, a little hexosamine and a small amount of tyrosine tightly associated with the collagen. Whether or not these substances are integral parts of the molecule, and so concerned in the fibrillogenesis, is still uncertain.

Reticulin: What does seem certain is that other connective tissue components are intimately involved in the architecture of the mature fibres. The problem of the nature of reticulin will serve to illustrate this point. Reticulin is a special kind of collagen visualized in the light microscope as fine isotopic fibres which show true branching, usually at right angles and with different staining reactions from ordinary collagens. It is most easily seen in relation to the basement membranes between connective tissue and epithelium and around muscle, nerve fibres, and similar structures. During the past few years these fibres have been shown to consist of 85 per cent protein, 4.2 per cent carbohydrate and 10.8 per cent bound fatty acids. (Table 1.) The protein is quite certainly collagen. The carbohydrate is curious in that it contains neither hexosamine nor uronic acid, nor was sulphate ester detected. Of the bound fatty acids some 95 per cent proved to be myristic acid and the remainder palmitic acid. It undergoes dissolution by collagenase but in some other respects, for example acid hydrolysis and autoclaving, it is much more stable than ordinary collagen. The association of collagen with other components has produced a special kind of fibre to subserve the needs of particular tissues. Before leaving the subject of reticulin it would seem desirable to distinguish this special form of collagen from other argyrophil fibres which still go by the name "reticulin." The most important example of these is the immature argyrophil collagen fibre to be found in areas of fibrillogenesis, whether embryonic or reparative. Although somewhat similar histologically, they do not have the special chemical make-up of the true reticulin fibre. General adoption of some descriptive term such as "reticulin-like" or even "reticular" for these latter kinds of argyrophil fibres would help to avoid further confusion.

Returning to ordinary collagen as it occurs in the tissues some further points of general interest deserve mention. Careful measurements have been made of the collagen fibrils in different tissues. It has been found that particular tissues have their own characteristic average fibril width, which differs from one tissue to the next. Furthermore these characteristic fibril thicknesses have been found to increase with age. Complementary to this is a decrease in the amount of interfibrillary ground substance as the body ages. During normal growth and development most tissues of the body show an increasing concentration of collagen with increasing size. During the active growth phase of early life the concentration increases sharply.

For example there is a sixfold increase in collagen concentration from the rat foetus to the young rat when expressed in terms of total weight increase. This early differential increase in collagen is unlikely to be related simply to ageing. It seems more likely that these massive increases with increasing size are determined by the requirements of mechanical stability in a body of increasing size.

The highest concentrations and most of the total body collagen occur in three tissue "components": in the skin, in tendon and fascia, and in bone and cartilage. The skin, in fact, may account for nearly half the total collagen in the body. In the parenchymatous viscera, which contain only small amounts of collagen, a substantial part of the collagen present is associated with the vascular tree.

Biogenesis of Collagen: A considerable number of studies have been made on the biogenesis of collagen using histological, electron-optical and biochemical methods, in embryo tissue, in tissue cultures and even *in vitro*. The course of events appears to be as follows.

The active fibroblast contains granular material in the cytoplasm which is known to include protein and mucopolysaccharide, and also alkaline phosphatase and cytochrome oxidase. There is recent evidence of the presence of a metabolically very active precursor collagen (hydroxyproline-containing protein) within the cell. By fractionation it corresponds to a "mitochondrial" portion of the fibroblast [*12*]. These granules probably represent the site of formation of a collagen subunit or molecule. This is secreted into the extracellular space where it begins to aggregate into a fibrous structure. It is invisible by light microscope at this stage but may be visualized by electron optics as fine fibrils, some appearing at the surface of the fibroblast. In embryonic tissue the cell surface of the fibroblast is not easily defined and in early embryos some fibrillar material may appear to be intracellular. This early fibril material is probably mostly extractable by neutral salt solutions and may correspond initially to tropocollagen. The fibres then continue to develop extracellularly by aggregation of fibrillar units. Later association with non-collagenous mucoprotin probably forms increasingly insoluble complexes. In this connection there is some evidence that chondroitin sulphate may be concerned with the stability of collagen fibres [*19*]. Incorporation of lipo-polysaccharide at an early stage of fibre formation will give the special modification of collagen termed reticulin.

Elastin: This second component of the fibrous system appears to be, in a mechanical sense, complementary to the collagens. It has much more elasticity than collagen but only about one-tenth its breaking strength. Seen in dissection of tissues in which it occurs in large amounts, e.g., the ligamenta subflava, it has a yellow appearance, contrasting with the pearl-white collagen. There are large amounts of it in the vascular system in which its mechanical properties are obviously desirable. Under the light microscope it is seen in a number of forms, branching fibres, sheet-like structures and debris material. The fibres are highly refractile and can be distinguished by selective stains, e.g., orcein.

Examination of elastin by x-ray diffraction reveals a pattern quite distinct from that of collagen, and there is no evidence of any periodicity under the electron microscope. The elastic fibres are quite remarkably stable and resist all the usual procedures employed for extracting collagen and other proteins from tissue. Indeed, elastin has been defined as the fibrous material remaining in the debris after all else has been removed! In addition it appears always to be intimately associated with true collagen in the body. According to Lancing [*23*] collagen fibrils can be liberated from the internal structure of the elastin fibre by treatment, first with sodium hydroxide and then with elastase. The difficulties of purifying elastin have presented a problem in research for many years, and it is only quite recently that substantial progress has been made. The present position may be summarized as follows.

Elastin appears to consist of two fibrous proteins, labelled α and β. They have a similar amino acid composition; in both hydroxyproline is low but there is rather more proline than in collagen and much larger amounts of valine. (Table II.) In this work Partridge, Davis and Adair [*31,32*] also found the elastin from different tissues to be very similar chemically. The α protein has a molecular weight of about 60,000 to 84,000, and the β component of about 6,000. These purified proteins contain not more than 0.3 per cent carbohydrate and insignificant amounts of ester-bound sulphate, although it is known that the native elastic fibres contain a substantial amount of a mucopolysaccharide similar to chondroitin sulphate.

What is the structure of the native elastic

fibres? Lancing and co-workers [*23*] studied the dissolution of elastic fibres by elastase using electron optics. They concluded that the fibres (about 5 microns in diameter) were built up from many minute threads of fairly uniform thickness. These threads are about 25 mμ in diameter and are apparently the elementary units, at any rate in ligamentum nuchae. Varying numbers of these threads are twisted together to form four thicker fibrillar components, each about 1 micron in diameter. The whole structure appears to be cemented together and coated with a matrix material. Further extensive evidence of the dual structure of the elastic fibre has been obtained by Hall, Reed and Tunbridge [*14*]. On the present evidence the elastic fibre would appear to be a two-phase system. The inner fibrillar core consists of the α protein whilst the outer sheath consists of the β protein in association with an acid mucopolysaccharide. These two protein phases, the α and β, are believed to be formed from identical protein units. This picture of the elastic fibre takes no account of the undoubted presence of protein-bound unsaturated fatty acid, and a yellow pigment associated with it. Both the unsaturated lipid and plasmogen are tightly bound, and may well contribute to the great stability of the elastic fibre. The fibres also appear to contain about 0.01 per cent phosphorus which resists extraction and is presumed to be in phosphoprotein combination.

Recently evidence has been presented suggesting that elastin is a member of the collagen family [*13,14,21*], and that it does not in fact constitute a quite different structural protein. This envisages elastin arising in the tissues from altered or degraded collagen units. Certainly no connective tissue cell has as yet been defined as an "elastoblast," and collagen is intimately associated with the elastic fibres. However, the differences in amino acid patterns between the two structural proteins will require further exploration in this context, to mention only one difficulty. It is an interesting approach to the problem of elastin and should assist future research. No satisfactory experimental model in which elastin can be produced has as yet been devised. What must seem to be required is a system of connective tissue under active metabolic stimulation, the whole being subjected to pulsating stress. Ordinary repair tissue and tissue cultures either do not produce elastin at all or only very slowly, in the case of repair.

TABLE II

AMINO ACID ANALYSIS OF ELASTIN AND THE PROTEINS DERIVED FROM IT (PARTRIDGE ET AL. [*31,32*])

Amino Acids	Elastin from Ligamentum Nuchae	α Protein	β Protein	Elastin from Aorta
	(in gm. per 100 gm. dry weight protein)			
Glycine	27	23.2	29.0	26.2
Alanine	23.1	24.5	20.8	21.6
Leucine	8.9	8.3	8.1	9.5
Isoleucine	3.7	3.0	3.8	4.3
Valine	17.0	15.5	20.6	18.0
Serine	1.0	0.89	0.85	1.5
Threonine	1.2	1.1	0.98	1.7
Proline	12.8	12.3	13.8	14.5
Hydroxyproline	1.6	1.5	Trace	1.7
Phenylalanine	6.2	.....	4.6	6.2
Tyrosine	1.2	1.6	1.1	2.6
Methionine	Trace	Trace	Trace	0.53
Histidine	0.09	0.03	0.05	0.31
Arginine	1.2	1.2	0.93	1.9
Lysine	0.54	0.56	0.48	1.2
Aspartic acid	1.1	0.66	1.0	2.1
Glutamic acid	2.6	2.6	2.1	3.9
Cystine	.....	0.40	.....	.....

The Non-fibrous Proteins. Plasma Proteins: At least half, and probably more, of the total plasma protein of the body is outside the circulation. Exactly where in the body the extravascular protein is situated is still only partly known. Recently the use of Coons' fluorescent antibody technic and of radioisotope-labelled plasma proteins has provided some interesting information. So far as the connective tissues are concerned this information may be tentatively summarized as follows.

The amount of plasma protein found in various connective tissues varies considerably, as might be expected. Subcutaneous tissue contains some 4 per cent plasma proteins, skin about 1 per cent. Tendons, on the other hand, contain smaller amounts of plasma proteins; rat-tail tendon about 0.1 per cent and ox Achilles tendon about 0.25 per cent. The plasma proteins in skin probably account for about 25 per cent of the total extravascular plasma protein.

A large part of the soluble proteins extractable from connective tissue appears to be serum albumin [*16*]. In skin, some 95 per cent of the water-soluble protein may be characterized as albumin. But there is good evidence that only about 60 per cent of this albumin fraction is in fact serum albumin. The remainder appears to be a heterogeneous protein consisting mainly of a protein of albumin character. This latter

TABLE III
AMINO ACID COMPOSITION OF CARTILAGE MUCOPROTEIN COMPARED WITH OX BONE COLLAGEN (PARTRIDGE ET AL. [33])

Amino Acids	Cartilage Mucoprotein Prepared by		Ox Bone Collagen (Eastoe [106])
	Acid Hydrolysis	Alkaline Hydrolysis	
	(in gm. per 100 gm. dry weight original material)		
Hydroxyproline	0.04	Nil	14.1
Aspartic acid	7.96	7.3	7.1
Threonine	3.19	3.27	2.52
Serine	3.25	2.65	4.24
Glutamic acid	10.0	12.4	11.9
Proline	6.05	7.86	14.7
Glycine	5.54	3.46	25.3
Alanine	4.24	4.11	10.5
Valine	4.49	4.54	2.65
Methionine	1.23	1.22	0.80
Isoleucine	3.91	3.50	1.73
Leucine	7.69	7.73	3.93
Tyrosine	4.38	4.54	0.56
Phenylalanine	5.12	7.42	2.88
Histidine	.....	1.83	0.96
Lysine	.....	3.50	4.11
Arginine	.....	2.3	9.2
Glucosamine	.....	0.61	.....
Galactosamine	.....	0.56	.....
Unknown amino sugar	.....	0.33	.....

albumin fraction may be produced within the skin itself. Information about the globulin fractions is very limited. There is little doubt that connective tissue contains serum globulins in addition to serum albumin, but probably not in the same proportions as in blood. Electrophoretic evidence suggests the presence of significant amounts of other proteins associated with the serum globulins, and some of these are probably synthesized in the connective tissues.

Other Connective Tissue Proteins: The existence of proteins other than fibrous or plasma proteins in connective tissue has been suspected for a number of years. Beyond the bare fact of their probable existence almost nothing was known of their composition until recently, when one of them was isolated from cartilage. In 1954 Shatton and Schubert [40] discovered a hitherto unrecognized protein component in combination with chondroitin sulphate in cartilage. This protein has been subjected to more detailed analysis by Partridge and Davis [33]. From these studies it would seem that the greater part of the chondroitin sulphate in cartilage exists as a mucoprotein complex, the protein being non-collagenous. This protein contains no hydroxyproline and the whole amino acid pattern is quite different from collagen. (Table III.) Of particular interest is the presence in this protein of 4.5 per cent tyrosine. Furthermore, about a third of this material appears to contain nitrogenous and non-nitrogenous components other than amino acids. Glucosamine, which cannot have come from the chondroitin sulphate, was present in all the purified samples of the protein. It seems probable from this work that the integrity of native cartilage depends upon linkages between collagen and this protein-polysaccharide macromolecule. Nothing is known of the nature of these linkages but Muir [29] has found that on breaking the polysaccharide-protein macromolecule by papain the amino acid serine is found, still attached to the polysaccharide.

These studies on the chondroitin sulphate-protein macromolecule are important in the whole context of connective tissues; important because they demonstrate that in at least one connective tissue, cartilage, the mucopolysaccharide probably does not exist in the free state *in vivo*. The integrity of cartilage would seem to depend upon bonds between this macromolecule and collagen, and not between the chondroitin sulphate and collagen. There have been a number of reports of a tyrosine-rich material being found, in extracts of connective tissue, in association with polysaccharide. Such material has been found in bone, skin and other connective tissue. This protein material seems always to contain tyrosine in large amounts, and is always found in association with carbohydrate, although the carbohydrate moiety may or may not contain uronic acid or hexosamine. The amounts of these mucoproteins are usually small in relation to the amount of fibrous protein, forming some 3 to 5 per cent of the total collagen or elastin fibre. Although they are present in most connective tissue only in small amounts they may well prove to have great biological significance. Evidence is slowly accumulating of their importance in the maintenance of the

final structures of living connective tissue. The isolation of specific enzymes which attack these mucoproteins should greatly facilitate research [*2*].

THE POLYSACCHARIDES OF CONNECTIVE TISSUE

For practical discussion it is convenient to classify the connective tissue polysaccharides under two broad heads: (1) acid mucopolysaccharides and (2) all others. Only the acidic mucopolysaccharides will be discussed here. Group two includes neutral or acid mucoids and glycoproteins which certainly occur in connective tissues. Many of these occur in association with the plasma proteins, but it seems probable that some of them have connective tissue origins. Unfortunately no defined compounds have been isolated and discussion of them must await future research. Speaking of the role of polysaccharides in relation to connective tissue fibre synthesis and general activities, Astbury [*1*] made the following pertinent remark, " . . . these polysaccharides are among the most self-effacing molecular collaborators imaginable, the nearer their job to completion, the fewer the stoichiometric signs that they ever had anything to do with it. In the end they are rather like the Cheshire cat—only their grin remains."

The acid mucopolysaccharides are divisible into two groups on the basis of whether they are sulphated or not. The non-sulphated group includes hyaluronic acid and chondroitin. The sulphated mucopolysaccharides include the three chondroitin sulphates A, B and C, heparitin sulphate and keratosulphate. This list comprises the mucopolysaccharides from connective tissue which have been isolated, and in some measure characterized. It seems more than likely that the list will be extended in the near future.

Hyaluronic Acid: This appears to be present in most connective tissues although the amount varies greatly. It is particularly abundant in umbilical cord, synovial fluid, vitreous humour and in some mesodermal tumours and rheumatic nodules. It appears to be formed by the least differentiated fibroblast. It is reported absent in hyaline cartilage and in cornea. Its structure is given as an unbranched polymer of a disaccharide repeating unit, N-acetyl hyalobiuronic acid, joined by β-4-glucosamidic bonds. It appears to have constant chemical properties as isolated from different sources, but there are considerable variations in molecular weight and degree of polydispersity, which determine its physical properties in the connective tissue. The viscosity of hyaluronic acid as it occurs in the body, however, is also influenced, in some situations at least, by association with protein. Blumberg and Ogston [*4*] have shown that hyaluronic acid exists as a complex with protein in human synovial fluid, human umbilical cord and ox synovial fluid, and that this complex with protein is responsible for the observed non-Newtonian viscosity.

Chondroitin: This non-sulphated polysaccharide appears to be an isomer of hyaluronic acid in which the D-glucosamine is replaced by D-galactosamine [*9*]. Like hyaluronic acid it forms viscous solutions, gives a mucin clot on acidification and is digested by testicular and bacterial hyaluronidases at a rate similar to that of hyaluronic acid. It has been isolated only from cornea.

The Chondroitin Sulphates: These are composed of equimolar concentrations of N-acetyl D-galactosamine, uronic acid and sulphate. Three members of this group have been distinguished on the basis of solubilities, optical rotation and enzymatic hydrolysis, and labelled A, B and C. A and C are closely related and the uronic acid in both is D-glucuronic acid. Chondroitin sulphate B differs in several respects from A and C. Its optical rotation is about -60 degrees, its calcium salt is less soluble than either A or C, and it is completely resistant to hydrolysis by testicular or bacterial hyaluronidase. It is also different chemically in that the uronic acid present is a mixture of D-glucuronic acid and L-iduronic acid [*27*]. It is not known whether the occurrence of these two uronic acids means that chondroitin sulphate B is a mixture, or whether it is in fact one polysaccharide. Its chemical and physical properties, however, as obtained from various sources and by different methods were closely similar.

According to Meyer, β-heparin obtained from beef lung, and a fraction obtained by Smith and Gallop [*43*] from hog stomach, are identical with chondroitin sulphate B.

The distribution of these chondroitin sulphates in the tissues varies considerably. From the limited information available it would seem that chondroitin sulphate A occurs in cartilage, bone, cornea, aorta and in ligamentum nuchae. Chondroitin sulphate C also occurs in cartilage and in umbilical cord, tendon and nucleus

pulposus. Chondroitin sulphate B has been isolated from skin, tendon, heart valves, aorta and ligamentum nuchae. Some further remarks on the distribution of polysaccharides will be made later.

Keratosulphate: This is a sulphated mucopolysaccharide which is free of uronic acid. It is composed of equimolar amounts of N-acetyl glucosamine, galactose and sulphate. Its structure is unknown. Keratosulphate has been isolated from cornea, in which it constitutes some 50 per cent of the total polysaccharide. It has also been found in growing bone in small amounts, but appears to be present in substantial amount in the nucleus pulposus.

Heparitin Sulphates: Mucopolysaccharide fractions have been isolated from bovine aorta and from human amyloid tissue which are composed of equimolar amounts of glucosamine, uronic acid and sulphate. These fractions have a strong positive optical rotation and are resistant to testicular hyaluronidase. They exhibit high carbazole and low orcinol values relative to the hexosamine content, as does heparin. Their structure is unknown.

Low-Sulphate Fractions: Mucopolysaccharides which appear to be chondroitin sulphate A or C, but with much lower sulphate content, are found in some tissues. Meyer reports them to be abundant in growing bone. The sulphate deficiency does not appear to be due to loss of sulphate during preparation. I have reason to believe that similar low-sulphate chondroitin sulphates occur in a situation in which active connective tissue growth is being stimulated by carrageenin. Whether or not these low-sulphate mucopolysaccharides are precursors of the chondroitin sulphates A and C is unknown.

The Polysaccharide Content of Some Connective Tissues: Estimates of the total amounts of polysaccharide present in connective tissues are dependent on severe limitations imposed by the analytical methods available at present. Hexosamine and uronic acid values in hydrolysates of connective tissues in which the concentration of polysaccharides is small, relative to protein, are notoriously unreliable. As an example of this difficulty Boas [*5*] found 0.4 per cent dry weight total hexosamine in rat subcutaneous tissue. Of this, only one-third could be specifically accounted for by a mucopolysaccharide. Almost 50 per cent of the total hexosamine was found to be associated with ground substance proteins, which were electrophoretically similar to plasma proteins. More precise information may come from the recent introduction of methods of extracting polysaccharides by means of proteolytic enzymes such as papain and ficin, and their selective precipitation by means of cetyl pyridinium chloride [*39*].

The best published information on mucopolysaccharide distribution comes from Meyer et al. [*27*]. Tendon and heart valves were found to contain about 0.5 per cent by weight of acid polysaccharide, in terms of dry, defatted tissue. Chondroitin sulphate B and C were present in about equal quantities and hyaluronic acid was found as a minor component. Adult skin contained about 0.3 to 0.4 per cent acid polysaccharide, again as dry, defatted weight. The major component in skin was chondroitin sulphate B, followed by hyaluronic acid. Chondroitin sulphate C was found, but in very small amounts. At least one other unidentified acid polysaccharide was present. Embryo skin was found to contain much higher amounts of total polysaccharide, and it is most interesting that the main polysaccharide in the young skin was hyaluronic acid. The ratio of chondroitin sulphate B to hyaluronic acid in adult skin was 1.25, in embryo skin 0.20. It is this kind of observation which gives force to the argument that hyaluronic acid appears to be a product of the least differentiated fibroblasts. Ligamentum nuchae also contains chondroitin sulphate B and hyaluronate as major components; the chondroitin sulphate B/hyaluronic acid ratio was 1.5. A third component which was found appeared to be chondroitin sulphate A.

Abdominal aorta was studied from young cattle and human subjects aged about sixty-four years. The total polysaccharide was about 1 per cent in the young cattle and 0.8 per cent in man. In the bovine aorta about 43 per cent was chondroitin sulphate A, 23 per cent hyaluronic acid, the remainder being made up of chondroitin sulphate B and heparatin sulphate in approximately equal proportions. The polysaccharide pattern in human aortas from subjects in their sixties was strikingly similar to that in young cattle, with the exception of hyaluronic acid which contributed much less than 10 per cent of the total.

From Meyer's data it is clear that chondroitin sulphate B is not a constant component of elastic tissue, but that all three chondroitin sulphates are found in collagenous tissue. It would indeed be a major advance if differences

in type and distribution of the acid mucopolysaccharides could be given histological interpretation.

A little information is available on the distribution of polysaccharide in connective tissue in relation to collagen. The polysaccharide/collagen ratio obviously varies greatly. In skin and cornea, polysaccharide appears to be less than 1/20th the amount of collagen. Cartilage has about equal amounts of polysaccharide and collagen in terms of dry weight. On the other hand synovial fluid has no collagen and hyaluronic acid only, and there is a high polysaccharide/collagen ratio in vitreous humour.

THE WATER IN CONNECTIVE TISSUE

Any consideration of connective tissue is incomplete without at least some brief mention of the water phase. In all connective tissues other than bone, water forms by far the largest single component. Most connective tissue contains upwards of 70 per cent water by weight. The role of the connective tissue components in influencing the diffusion and exchanges of electrolytes, and other solutes, is still largely unknown. But at least it is now recognized that they are not just an inert sieve, through which electrolytes and metabolites pass from the circulation to the cells. The few remarks to follow must serve to emphasize the great importance of this dynamic aspect of the connective tissues.

How much of the water in connective tissue is freely moving, that is unbound and in fluid form? The answer is, probably very little, at least in normal health. In skin there appears to be no interstitial water in fluid form [*34*]. When pathological changes take place, however, free fluid may appear in quantity, as in cardiac and nephrotic oedema. It seems likely that the water in connective tissue is bound to the mucopolysaccharide-mucoprotein-fibrous protein complexes. The forces binding this water are probably similar to those binding water of crystallization. But the system is obviously a complex one and there is a wide range of degrees of water binding. This concept does not conflict with rapid metabolic processes involving exchange of water and electrolytes. Indeed it should help future understanding of the movements of body water and its solutes, about which there is still little precise information. The time required for equilibration between various plasma components and these same components in the various connective tissues must be subject to wide variation. A striking example of this concerns plasma albumin. Humphrey, Neuberger and Perkins [*16*] have found that the time required for complete equilibration between I^{131}-labelled albumin in plasma and in skin of the rabbit requires some six to eight days.

When connective tissue swells there is observed swelling of fibroblasts, and increased hydration of ground substances and collagen fibres. It seems probable that the increase in hydration of the polysaccharide-mucoprotein occurs before the swelling of the collagen fibres. These changes in water binding would seem to be initiated by alterations in the energy balances of the tissue. The over-all structure of a connective tissue probably requires energy to maintain its equilibrium. Very little is known about the part played by individual connective tissue components in controlling hydration. It is known, however, that in tissues which are highly hydrated the prominent polysaccharide is hyaluronic acid. Where sulphated polysaccharides wholly or largely replace hyaluronic acid there is a lower degree of hydration. The ion binding properties of connective tissue polysaccharide complexes must also be involved. But this aspect, and the whole position of the fluid phase in connective tissue, is at present obscure.

CONNECTIVE TISSUE METABOLISM

The Fibrous Proteins. Collagens: The collagen in tissue is divisible into three biochemical fractions on the basis of differing solubilities. The first of these is neutral salt-soluble and may also be extracted by means of slightly alkaline buffers. This fraction presumably includes any tropocollagen present and newly formed fibril material in general. The amount of neutral salt-soluble collagen in connective tissue is always small in relation to the total collagen, except in very actively growing tissue. It contains the precursor collagen material.

The second fraction is acid-soluble collagen which should be defined as the collagen fraction soluble in acid citrate buffer. This is not quite the same as collagen extractable by dilute acetic acid. Collagen extracted by dilute acetic acid has no definable end point; prolonged extraction in series continues to remove further small amounts of collagen. Acidic citrate buffer, however, does seem to give a limited fraction of the

collagen. It is a much larger fraction than the neutral salt-soluble collagen and is readily obtained even from adult connective tissue.

The last fraction is insoluble collagen, i.e., all the collagen which has resisted extraction by the first two procedures mentioned. As would be expected it forms by far the largest fraction of collagen, especially in older connective tissue.

The metabolism of these three fractions of collagen has been studied in some detail and, despite the obvious limitations of the fractionation procedures, we now have a rather clearer picture of collagen metabolism.

Where there is active connective tissue growth, neutral salt-soluble collagen can be extracted which shows a high rate of metabolism. It would seem to contain a precursor collagen with a "half-life" of the order of two days, i.e., a faster rate of metabolism even than liver protein. It has been studied in connective tissue of young animals [*18*], and in connective tissue stimulated into activity by carrageenin [*20*]; both situations in which sufficient definable material can be obtained for biochemical procedures. In adult connective tissue it seems probable that this metabolically active fraction must occur, in tiny amounts, in localized sites in which new synthesis of collagen is required. Otherwise it is difficult to understand how the metabolically relatively inert, insoluble collagen always seems to contain some small amount of labelled radioactive tracer.

The acid-soluble fraction of collagen does not show the rapid rate of metabolic turnover seen in the neutral salt fraction. It shows a higher rate of incorporation of radioactive tracer than does insoluble collagen but, on the available evidence, the rate of loss of isotope is slow. It may represent an intermediary collagen, in the metabolic sense, between the labile precursor collagen and the fully formed stable collagen fibres. The evidence suggests this but is by no means conclusive. This material has been called procollagen by Orekhovitch [*30b*] and is claimed to be a precursor collagen. Whatever its position is in the metabolic scale, it is certainly not a precursor collagen in the accepted "Western" definition of that term.

The greater part of collagen, the insoluble collagen, is metabolically inert [*30*]. It is so inert that it seems likely that replacement of the fully formed fibres occurs only intermittently in localized areas. All the metabolic studies to date on collagen have necessarily been carried out on gross mixtures of collagen fractions. We have no information at all about its metabolism in localized areas of tissue. But some small, localized, synthesis of collagen must occur in adult connective tissue to explain the observed incorporation of radio-tracer. The amount of radio-tracer (^{14}C-labelled glycine) incorporated into the mature collagen fibres is very small. But it does occur, and then appears to remain in them for a very long time.

There is at least one situation in the body in which synthesis and resorption of collagen occur rapidly under physiological conditions. This is in the pregnant uterus [*18*]. Here collagen increase closely parallels the increase in total weight, and new collagen must be synthesized quite rapidly. Following the termination of pregnancy there is a very rapid removal of collagen. The metabolism of this interesting situation has not been studied in detail. The only artificial system which produces collagen rapidly in quantity, and later results in its rapid removal, is the granuloma stimulated by carrageenin, a polygalactose [*20,35*]. The controlling mechanisms in the two cases are quite different, but the course of events as regards collagen formation and removal are oddly similar.

In massive tissue wasting, for example in a whole hind limb [*41*], removal of collagen does occur, but it is very slow by comparison with the postpartum uterus or carrageenin granuloma.

Elastin: There is very little information about the metabolism of elastin. So far as the aorta of adult rats is concerned the elastin protein appears to be relatively metabolically inert, behaving similarly to bone collagen in adult rats [*42*]. This metabolic inertia of elastin protein would be in agreement with the concept of elastin as a special modification of collagen. Labella [*22*] has raised the possibility that not all the constituents of the elastic fibre are metabolically inert. It would appear that protein-bound phosphorus is in fact metabolically active. It may well be that constituents of the outer sheaths of elastic fibres are metabolically active, but this is a different thing from saying that elastin protein is metabolically active. The whole subject of the metabolic activity of elastic fibres, is worthy of further investigation.

The Mucopolysaccharides. Biosynthesis: The biosynthesis of the mucopolysaccharides has been the subject of intensive study recently. The main pathways of synthesis from precursors, the uridine diphosphate glycosides, are becoming

clearer. More is known about hyaluronic acid synthesis than about the sulphated polysaccharides. In the case of hyaluronic acid the precursors are glucosamine and glucuronic acid from which uridine diphosphate acetylglucosamine (UDPAG) and uridine diphosphate glucuronic acid (UDPGA) can be synthesized. From the UDPAG and UDPGA low molecular weight hyaluronic acid can be polymerized using cell-free extracts of Rous sarcoma. In the case of the sulphated mucopolysaccharides the point at which the sulphate is introduced into the molecular structure is of great interest in the interpretation of metabolic studies using ^{35}S-labelled sulphate. All the evidence accumulating seems to point to incorporation of the sulphate into a sugar monomer, rather than into a preformed polymer such as chondroitin. This would mean that the use of ^{35}S-labelled sulphate in metabolic studies does in fact reflect complete synthesis of the mucopolysaccharide polymers. It is interesting, in the evolutionary context, that simple organisms seem unable to synthesize sulphated polysaccharides. The ability to do this must have been an important step forward in the evolutionary process.

Metabolism: Information about the metabolism of the connective tissue mucopolysaccharides derives mainly from two methods of study. In both cases $Na_2{}^{35}SO_4$ is used as the radioactive tracer. The first method involves the location of "bound" sulphate in tissue sections by means of autoradiography. In the second method $^{35}SO_4$-labelled polysaccharides are extracted from the tissues biochemically. Both methods have contributed to our present rather limited knowledge of the metabolism of the mucopolysaccharides. Neither method does more than indicate the probable rate of metabolism of a mixed population of sulphated polysaccharides. No information about the rates of metabolism of individual sulphated mucopolysaccharides is as yet available, but developments in methods of biochemical separation should enable the second method to give more definitive information.

Chondroitin Sulphates: The rate of metabolism of mixed chondroitin sulphates in rat skin and rat cartilage was found to be ten days and sixteen days, respectively [*7,8*]. These over-all rates of metabolism have been subsequently confirmed by the author and other workers in several animals, although the rate of turnover in skin appears often to be rather faster, eight days or so. The rate of metabolism in skin, then, would appear to be about twice the rate in cartilage. Since none of the metabolic studies to date differentiate between chondroitin sulphates A, B and C, and other sulphated polysaccharides which may be present, it is difficult to interpret this difference. It may well be that this difference in rates of metabolism of the total sulphated polysaccharides reflects differing metabolic activity as between chondroitin sulphate B and chondroitin sulphates A and C.

The methods of extraction employed in biochemical studies of mucopolysaccharides, to date, result in an end product which is probably the most stable, and therefore the least metabolically active, mixture of components. There is some indication that more active "metabolic fractions" may exist in the tissues. Mixed sulphated polysaccharides from granulation tissue induced by carrageenin injection [*19*] show quite different rates of metabolic activity, according to the method of extraction. Polysaccharide extracted by neutral salt solutions shows a high rate of metabolic activity. The fraction extracted next, by papain digestion, and containing the bulk of the tissue polysaccharide, has a metabolic rate somewhat faster than that in normal skin, but much less than the neutral salt fraction. A residue sulphate fraction appeared to have a very slow rate of metabolism. The biochemical identities of these fractions are unknown.

Using ^{14}C-labelled acetate and glucose, Schiller, Matthews and Dorfman [*36*] have found the turnover rate of mixed sulphated polysaccharides in skin to be about nine days. This suggests that measurements of sulphated polysaccharide metabolism by means of ^{35}S is a reliable measure of total polymer turnover, since the two methods are in good agreement.

Hyaluronic Acid: Very little work has been done on the metabolism of hyaluronic acid. From the work of Schiller, Matthews and Dorfman [*36*] it seems clear that hyaluronic acid in skin has a much faster rate of metabolism than the sulphated polysaccharides. Indeed, the rate would seem to be some three times as fast as the sulphated polysaccharides, the "half-life" being of the order of two days. This high rate of metabolism of the hyaluronic acid compared with the sulphated polysaccharides implies quite different dynamic rôles for the two groups of mucopolysaccharides. It is perhaps not surprising that the sulphated polysaccharides, being more intimately associated with the relatively stable fibrous proteins, should be the less

metabolically active of the two groups of mucopolysaccharides.

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Clinicopathologic Conference

Jaundice, Fluctuating Consciousness, Polyuria, Fever and Death

STENOGRAPHIC reports, edited by Lillian Recant, M.D. and W. Stanley Hartroft, M.D. of weekly clinicopathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine, Preventive Medicine, and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

A FORTY-SEVEN year old white male physician (L. E.) was admitted to Barnes Hospital for the first time on June 17, 1958. He died on July 9, 1958.

His chief complaint was jaundice of one day's duration.

The patient had always been in excellent health until approximately seven days prior to admission at which time he noted the onset of malaise and intermittent severe muscle pain associated with a temperature elevation to 101°F. Because of the persistence of these symptoms he was treated with penicillin and streptomycin. Four days prior to admission a roentgenogram of the chest was interpreted as showing "viral pneumonia," and a white blood cell count was said to have been 5,200 per cu. mm. with a "left shift." One observer had noted a transient episode of confusion. The next day there was a spontaneous defervesence of the temperature elevation, transient appearance of marked anorexia, and emesis on one occasion. Twenty-four hours later the patient noted the onset of jaundice. On the day of admission, he complained of severe, sharp, persistent epigastric pain without radiation. There was no accompanying nausea or vomiting at that time. The patient denied any change in the color of his stool or urine. Diarrhea and weight loss were also denied. A loss of his usual desire for tobacco was apparent from the onset of the present illness.

The patient had had no known exposure to persons with jaundice. He had not received any blood transfusions. Three months prior to admission, the patient incurred a laceration on his finger while performing surgery at a tuberculosis hospital on a patient who subsequently died with jaundice which was attributed to a new antituberculous drug.

The past history, family history and review of systems were non-contributory.

Physical examination revealed the following: pulse, 106 per minute; respirations, 20 per minute; temperature, 36.7°C.; blood pressure, 140/60 mm. Hg.

The patient was well nourished, obviously icteric and complaining of acute abdominal pain. The skin showed frank icterus. Over the posterior upper thorax there were many small superficial venules. No significant enlargement of the lymph nodes was noted. The scleras were icteric. There was no conjunctival injection. The fundi were within normal limits. There was injection of the posterior pharynx. No abnormal physical signs were detected in the chest. Examination of the precordium revealed the point of maximal impulse to be in the sixth intercostal space in the mid-clavicular line; the rhythm was regular, and there were grade 2 apical and basilar blowing systolic murmurs. One observer noted a questionable precordial friction rub. The abdomen was soft and symmetrical with moderate epigastric tenderness and voluntary guarding. The liver edge was palpable 3 cm. below the right costal margin, but was not tender. The spleen could not be felt. There were no signs of ascites or of peripheral edema. Neurologic examination was normal except for the sensorium which was clouded, and response was inadequate in all areas of questioning.

Laboratory data on admission were as follows: hemoglobin, 15.1 gm. per cent; white blood cell

count, 15, 150/cu. ml. Differential: band forms, 17 per cent; segmented forms, 54 per cent; lymphocytes, 27 per cent; monocytes 2 per cent. Platelets were normal. Urobilinogen was 0.36 mg. per cent; bile, negative. 10 to 15 white blood cells were seen per high power field in the centrifuged sediment. The stool was guaiac positive, and benzidine was 3 plus. (Table I.)

The heterophil agglutination and agglutination tests for Salmonella typhosa, Salmonella groups C and E were negative. Roentgenographic examination of the chest revealed a calcified tuberculous scar at the upper pole of the left hilum, and diffuse prominent markings throughout both lungs of indeterminate significance. The changes of reflex ileus were seen in roentgenograms of the abdomen. An electrocardiogram showed an abnormal form of ventricular complex suggestive of anterior myocardial ischemia although pericarditis could not be excluded.

The patient was seen by a surgical consultant who believed that there were no signs suggestive of an acute surgical condition of the abdomen and advised against any definitive surgical procedures. Because of excruciating epigastric pain the patient was given 50 mg. per cent of Demerol® and there was prompt relief of pain. On June 18, 1958, the patient was noted to have become more confused and irrational. He was disoriented as to time and place, and was somnolent. The neurological examination was otherwise within normal limits. Because he was thought to be in impending hepatic coma, a regimen was started including protein restriction, vitamin supplementation, enemas, administration of broad-spectrum antibiotics, hydrocortisone, and parenteral fluid therapy of 10 per cent glucose and water. During the ensuing three days his mental status was that of intermittent mental confusion, with emotional lability, and somnolence. The persistently prolonged prothrombin time did not respond to administration of vitamin K_1 oxide; therefore he was given transfusions of fresh plasma repeatedly.

On June 20, the patient had a twenty-four-hour urine output of 7,965 ml.; the specific gravity was 1.004 and the urinary sodium excretion was 2 mEq./L. A urine culture was negative. The urine pH during this time ranged between 6.0 and 7.0. For the succeeding five days, the urine volume varied from 4 to 7 L./twenty-four hours; appropriate replacement therapy was required. Examinations of the urine for reducing substances during this time revealed no abnormalities. Repeat urinary sodium determination on June 23 was 2.5 mEq./L., and urinary potassium was 9.2 mEq./L. Supplemental potassium therapy was also begun at this time.

On June 23, the arterial blood ammonia was 146 gammas per cent (normal 70 ± 20 gammas per cent). A flapping tremor was detected by one observer for the first time. Definite clinical improvement had been noted by June 26 with mental clearing, an improved appetite, and desire for food. The liver edge was felt 2 cm. below the right costal margin and was slightly tender. On June 26 the hydrocortisone was replaced by prednisolone, 8 mg. four times a day. By July 2, the patient had become verbose and irrational. Prior to and during this time he complained frequently of epigastric burning pain. Repeat stool examinations revealed a constant trace of occult blood by the benzidine reaction. It was believed that the patient probably had a toxic psychosis secondary to steroid therapy, and slow withdrawal of the steroids was attempted. During the following three days, there was progressive deterioration with the persistence of a semicomatose, irrational state. Numerous small purpuric and petechial areas appeared over dependent portions of the body.

On July 8 the patient was unresponsive. The abdomen was distended and tympanitic; there were no bowel sounds. A stool specimen contained gross blood, although the hematocrit remained stable.

Signs of ascites were apparent at this time although there was no peripheral edema. During the last four to five hours of life, the patient became markedly tachypneic with a respiratory rate of 45 per minute. Examination of the lungs revealed no cause for these changes. He was given digitalis, oxygen, and aminophylline without apparent benefit. He died at 5:00 A.M. on July 9.

CLINICAL DISCUSSION

DR. EDWARD REINHARD: This physician's finger was punctured by a needle during an operation performed at a tuberculosis hospital. The patient who was being operated upon had advanced tuberculosis and subsequently died with jaundice some months later; he was being treated with "a new anti-tuberculosis drug known to be capable of producing jaundice and death." We have no significant infor-

TABLE I
LABORATORY DATA

Blood Tests	June													July							
	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8
Na (mEq./L.)	.....	.....	137	131	133	132	133	137	139	135	133	135	..	138	.....	....		131	.....	126	138
K (mEq./L.)	.....	.....	3.3	3.2	3.8	3.3	3.0	3.4	4.2	3.9	4.0	4.1	..	5.1	.....	....		4.3	.....	3.3	4.4
Cl (mEq./L.)	.....	.....	97	91	93	89	88	88	91	91	94	96	..	94	.....	....		89	.....	85	102
CO_2 (mEq./L.)	.....	28.5	27.9	32.6	35.6	35.2	31.5	40.2	38.8	33	31.6	34	..	30.7	.....	....		25.4	.....	31.3	32.7
Bilirubin direct/indirect (mg./100 ml.)	5.0/5.8	6.4/9.2	..	..	..	6.2/11.8	8.7/9.3	..	.....	8.7/10.8	..	....	..	..	14/22.4	....		8.8/18.4	12.8/20.8	..	....
Proteins, albumin/globulin (gm./100 ml.)	4.4/2.2	4.2/2.9	..	..	..	3.7/3.1	.....	..	3.4/3.4	.....	..	....	..	..	3.3/3.4	....		.....	3.2/3.4	..	....
SGOT*	430	.....	..	..	..	.....	220	..	.....	.....	..	....	..	..	104	....		101	.....	..	112
SGPT†	2000	.....	..	..	..	.....	200	..	.....	.....	..	....	..	..	.....	....		.....	.....	..	....
Prothrombin time (%)	.....	10	..	20.5	..	25	.....	21	28	.....	29	29	..	21	24	..	29	22	.....	26	26
White blood cell (per cu. mm.)	.....	.....	..	..	..	.....	.....	..	.....	10,300	..	20,900	..	..	11,900	....		.....	.....	..	21,000
Cholesterol (mg./100 ml.)	.....	174	..	130	..	.....	192	203	.....	174	..	....	..	..	.....	....		.....	.....	129	....

NOTE: The non-protein nitrogen was normal; blood urea nitrogen ranged from 2 to 14 mg./100 ml.; cephalin cholesterol flocculation was 3 to 4 plus; thymol turbidity 8 to 10 units and alkaline phosphatase 6.6 to 9.1 Bodansky units.

* Glutamic-oxalacetic.

† Glutamic-pyruvic transaminase.

mation about the drug but I presume it was pyrizinamide.

Our patient, the physician, presented with a history of intermittent severe muscle pain and fever for seven days prior to admission. Penicillin and streptomycin therapy was begun two days after the onset of his symptoms. A roentgenogram of the chest is said to have shown pneumonia four days prior to admission. About this time there was transient mental confusion. His temperature returned to normal and anorexia simultaneously developed. One day before admission he became jaundiced. On the day of admission severe persistent non-radiating epigastric pain developed. From the onset of the illness the patient had a complete loss of his usual desire to smoke. Dr. Shank, does the loss of a previous enjoyment of smoking really have diagnostic significance?

Dr. Robert Shank: We find this observation useful diagnostically because of its frequent relationship with viral hepatitis. Cirrhotic patients, with manifestations of hepatic decompensation, can often be found sittng in bed smoking. On the other hand, any acute gastrointestinal illness may be associated with some abhorence of smoking.

Dr. Reinhard: Dr. Humphrey, will you discuss the roentgenograms?

Dr. Harvey Humphrey: On the day of admission an emergency examination was made for possible intestinal obstruction. There was gas throughout the small bowel and air-fluid levels were evident. The small bowel loops were not dilated; gas and fluid were also present in the stomach. These changes were considered to be caused by a reflex ileus. We see the same findings more commonly in enteritis. There certainly was no evidence of intestinal obstruction. Examination of the chest revealed that if pneumonia was present it was in the subliminal stage. The heart appeared to be normal in size and shape.

Dr. Reinhard: You recall that this patient had had a roentgenogram of the chest taken four days prior to admission which was interpreted as showing viral pneumonia. We do not know that this diagnosis was justified. However, Zimmerman and his collaborators reported a clinical study of 295 cases of viral hepatitis in soldiers. Of these patients, 194 had an onset with influenzal-type symptoms, namely chills, generalized aching, fever and headache; some had rhinorrhea; a few had sore throats and cough. Zimmerman states that of these 194 patients, 5 per cent had viral pneumonia on admission to the hospital. Dr. Goldman, was this association just a peculiarity of that particular epidemic? Did the two diseases merely coexist by chance, or is there a relationship between the pneumonitis and viral hepatitis?

Dr. Alfred Goldman: As far as I know there is no particular relationship between these diseases. Occasionally viral pneumonia has been reported in patients who have evidence of hepatic damage with jaundice. Of course, jaundice associated with severe pneumonia is not uncommon. We usually attribute that to a hemolytic process. At least we did when we used to see severe pneumococcal lobar pneumonia. I suspect that Zimmerman's findings relate to his particular epidemic.

Dr. Reinhard: If this patient did have pneumonia which subsequently cleared, the infection could have aggravated and intensified the course of viral hepatitis. Now, let us consider the primary diagnosis. Obviously, viral hepatitis is one prominent possibility. There are, however, a few clinical features that were not completely typical of viral hepatitis. Although vague muscular aching is common in severe generalized viral infections, repeated notes in this patient's chart emphasized that he had severe persistent muscle pain. I gather that it was greater than one would ordinarily expect to see in viral hepatitis. Is that correct, Dr. Shank?

Dr. Shank: Muscle pain occurred early and persisted. The calf muscles in particular were tender to any kind of pressure. As I recall, this was more marked on one side than the other. Myalgia and arthralgia are common prodromal symptoms of viral hepatitis. Some of us think that they may be more severe and more common in infection due to serum hepatitis virus than in infectious hepatitis. The persistence of myalgia in this patient seemed quite unusual and the greater tenderness on one side than on the other was of interest. Actually we wondered at one point whether or not there might be thrombophlebitis. However, there never was definite evidence for that diagnosis.

Dr. Reinhard: Epigastric pain, particularly when the patient is jolted, is common in viral hepatitis. However, at the time this patient was having epigastric pain, the liver edge was felt 3 cm. below the right costal margin and was specifically stated to be non-tender. One

wonders, Dr. Schatz, if his epigastric pain was the type one usually sees in viral hepatitis.

DR. BERT SCHATZ: The fact that the liver was not tender is unusual for any inflammatory lesion, particularly hepatitis when percussion over the liver almost always elicits tenderness. The combination of fever, epigastric pain, jaundice and ileus associated with leukocytosis, suggested a stone in the common duct. However, the laboratory data showed such marked impairment of liver function that an obstructive type of jaundice seemed improbable. I have no good explanation for the severe epigastric pain. It certainly was of an unusual variety for viral hepatitis.

DR. REINHARD: Would you comment briefly on the diarrhea? Dr. Humphrey has mentioned that the roentgenographic findings are compatible with enteritis or ileus.

DR. SCHATZ: Patients with infectious hepatitis may have diarrhea. Proctoscopic examination may show an inflammatory reaction.

DR. REINHARD: Dr. Garfinkel, I believe that you saw this patient. What was your impression?

DR. BERNARD GARFINKEL: My first impression was that certain of his manifestations were not entirely characteristic of infectious hepatitis. The epigastric pain was certainly atypical; this man was screaming with pain at a time when you could elicit no pain on hepatic palpation. We initially thought that this might be a surgical problem but subsequent events eliminated that possibility. Diarrhea at the time of admission was not a problem; it could have been caused by the antibiotics used in treatment.

DR. REINHARD: We have been discussing the clinical features that are not entirely typical of viral hepatitis. There are, however, many features which are strongly indicative of that diagnosis. Dr. Karl, will you summarize the evidence in favor of viral hepatitis and tell us whether or not you think that is really the correct diagnosis?

DR. MICHAEL KARL: The onset with an influenzal-type of picture is not uncommon, so that everything we have talked about is compatible, with the exceptions that muscle pain and epigastric discomfort were more prominent than one usually sees in viral hepatitis. A palpable liver associated with jaundice is certainly consistent with the diagnosis. There is certainly laboratory evidence of widespread hepatocellular disease: elevated transaminases, positive flocculation tests, and low prothrombin time.

DR. REINHARD: In addition to hepatic disease and fever, this patient had conjunctival injection. He had a leukocytosis, some form of renal disease, and a terminal purpuric and petechial rash. These findings could certainly be considered characteristic of Weil's disease. Furthermore, the fatality rate is considerably higher in Weil's disease with severe hepatic and renal involvement than it is in viral hepatitis. Dr. Moore, I wonder if you would comment on this diagnosis?

DR. C. V. MOORE: While you are perfectly right in giving serious consideration to the possibility that this was Weil's disease, there are some things which make one less receptive to the idea. For instance, in Weil's disease one would expect to find conjunctivitis, not just conjunctival injection. Furthermore, when the person with Weil's disease runs a fatal course, renal disease with azotemia usually develops; in our patient azotemia did not develop and that would make one suspicious. It is common for a chill to be present at the onset of Weil's disease, but that is not constant. If leptospiral infection were seriously considered, there are several diagnostic procedures that might have been undertaken. The urine and the blood might well have been injected intraperitoneally into guinea pigs or hamsters. One might have looked for the antibody to Weil's disease, since the patient lived long enough for an antibody to develop. One might also have considered taking a biopsy specimen of muscle, although with the patient's bleeding tendency I can understand why that was not done. With Weil's disease, however, areas of muscle necrosis are found. One other comment should be made about the recorded laboratory data; the urine bilirubin should certainly have been positive with such a high level of direct reacting bilirubin.

DR. REINHARD: In addition, Weil's disease is rare in this part of the country. When it does occur, it is found primarily in sewer workers, farmers, ditch diggers, and others who are exposed to rat excreta. Perhaps the most important reason for discarding the diagnosis of Weil's disease is the nature of the renal lesion. In Weil's disease there is a severe nephritis characterized by hematuria, proteinuria and casts, none of which was present. The patient did, however, manifest renal dysfunction. That was one of the most fascinating aspects of this case to me. He was obviously retaining salt. However, instead of the usually decreased urine volume,

found in severe hepatic disease with salt retention, this patient had polyuria amounting to between 4 and 7 L. of urine/twenty-four hours. Dr. Bricker, would you discuss the possible mechanisms that might account for this unexpected water diuresis?

DR. NEAL BRICKER: The existence of an unremitting diuresis generally indicates that the kidneys are failing to defend the integrity of the patient's body fluids. The exceptions to this, of course, would be found in the patient who is receiving an exogenous drug designed to induce diuresis, who is having a spontaneous diuresis secondary to unloading of edema, or who is receiving large amounts of fluid concurrently. The available information suggests that none of these three situations existed in this patient. In an attempt to classify the diuretic state, it is useful to analyze and evaluate the composition of the urine; as you have indicated there was virtually no sodium in the urine, despite volumes in excess of 7 L./day. This provides strong evidence that the sites of sodium reabsorption in the nephron were functioning in an intact manner. The fact that the urine contained large amounts of free water indicates that the diuresis was a water diuresis. That helps us to localize the lesion in the nephron. In the proximal segment, water reabsorption occurs passively following solute reabsorption and we know of no examples wherein free water (hypotonic urine) is delivered from the proximal tubule as a consequence of disease. Consequently, the lesion in this patient which impaired the diffusion of water out of the nephron was either in the distal segment, or in the collecting duct. The functional defect could be the result of intrinsic renal disease with alteration of the architecture of the nephron or it could be mediated by extrarenal factors. The two diagnoses which may be entertained are (1) a transient state of diabetes insipidus and (2) a reversible lesion involving the tubular epithelial cells. With respect to diabetes insipidus, it is conceivable that in a patient with a very prolonged prothrombin time and evidence of a generalized hemorrhagic diathesis bleeding could have occurred into the posterior pituitary. However, the short-lived period of diuresis would seem to mitigate against that diagnosis. The second possibility, that of intrinsic renal disease, would of necessity involve a lesion that effects either the distal tubule or the collecting duct, without producing evidence of generalized renal dysfunction. The one disease we consider in this situation is hypokalemic nephropathy. First let me briefly mention a point against the diagnosis and then I will attempt to defend it. There is no clear-cut history of diarrhea or of any other source of potassium loss preceding admission. Yet on the day that the diuretic state was first noted, the patient's potassium was 3.3 mEq./L. It is known from experimental observations in dogs and rats that polyuria can begin within two days of potassium deprivation. For the forty-eight-hour period preceding the apparent onset of polyuria, the patient was subjected to large doses of a mineralocorticoid which does produce potassium diuresis; moreover he received repeated enemas (in treatment of incipient hepatic coma) which could have depleted him of potassium. Finally there was a moderate amount of diarrhea presumably related to the administration of antibiotics. Therefore, the most likely diagnosis to explain the persisting but ultimately reversible water diuresis is early hypokalemic nephropathy.

DR. W. STANLEY HARTROFT: When did the diuresis let up? I have not been able to find that out.

DR. BRICKER: The diuresis apparently began on the third day of hospitalization; it was severe for five days thereafter, with urine volumes ranging from 4 to 7 L./day and then gradually diminished. May I add that there was a strong suggestion that the diuresis did not occur because of over-administration of hypotonic fluids.

DR. REINHARD: This patient was treated with large doses of potassium. Dr. Bricker, would you comment on his response to potassium? Was the response good enough to support a diagnosis of hypokalemic nephropathy?

DR. BRICKER: It is consistent with the diagnosis in that the diuresis subsided in association with the administration of potassium. One other point which supports the existence of potassium depletion is the fact that hypokalemic alkalosis did develop and responded to exogenous potassium administration.

DR. SOL SHERRY: Dr. Bricker, I was intrigued by the diuresis and the fact that the patient was confused almost from the onset of his illness. Although we would probably consider hepatic factors most likely to be responsible for the confusion, I wondered about the possibility of encephalitis? Could encephalitis produce a diabetes-insipidus-like picture as quickly as this?

DR. BRICKER: The usual abnormality in renal function associated with diffuse cerebral disease is a diuretic state, but characteristically the diuresis is one of both salt and water. As to whether or not compression of the posterior pituitary for a short period of time may occur in association with encephalitis and be responsible for a state of diabetes insipidus, I do not know.

DR. REINHARD: Dr. Daughaday, I would like to pursue the suggestion of a hemorrhage into the pituitary, producing diabetes insipidus. Would you comment on this possibility? The patient certainly had a severe bleeding tendency.

DR. WILLIAM DAUGHADAY: In order to make the diagnosis of diabetes insipidus, it is essential to show that the individual cannot concentrate his urine even in the presence of serum hyperosmolality. I am still intrigued by the fact that at no time during this period of diuresis do we have evidence of serum hyperosmolality. Although Dr. Bricker has in his own mind excluded the possibility of excessive fluid administration as a cause, the patient was certainly receiving 4 to 7 L. of fluid a day, and we are not clearly told whether or not he was dehydrated at the time.

DR. BRICKER: It is my impression that the onset of the diuresis preceded the administration of large volumes of fluid and indeed that the latter was necessitated by the polyuria.

DR. DAUGHADAY: I would put a true diabetes insipidus as my third choice. I am impressed by the alkalosis that developed in this patient and think that gives Dr. Bricker a very strong point. I do not think that a water polydipsia has been excluded as a possibility. Because the serum is continuously hypo-osmolar, diabetes insipidus is a difficult diagnosis to make. Actually the urine volume was only 4 to 7 L. which is less than a complete diabetes insipidus. We would, therefore, have to say that if he did have diabetes insipidus, it was a partial one. I personally believe that no lesion will be found in the pituitary.

DR. REINHARD: Could we have established this diagnosis by giving the patient Pitressin®?

DR. BRICKER: Had intravenous Pitressin been administered and a hyperosmolal urine been obtained, we could fairly well rule out hypokalemic nephropathy. But the clinical condition apparently did not justify anything other than maintaining a fluid intake in accordance with output.

DR. REINHARD: So you still believe that the basic lesion here was hypokalemic nephropathy?

DR. BRICKER: It is the best diagnosis on the basis of the assembled facts. Unfortunately, however, there is a strong possibility that there will be no residual histologic changes. The duration of the diuresis was only approximately one week and the vacuolization of the distal tubular cells may well have been reversed during the ensuing period of potassium administration.

DR. REINHARD: Dr. Levy, some observers thought this patient had a steroid psychosis. Others thought that the changes were due to hepatic coma. The possibility of a cerebral hemorrhage was also considered. Dr. Sherry has raised the additional question of encephalitis. Would you comment?

DR. IRWIN LEVY: The fluctuating consciousness appears to be most compatible with hepatic coma. There is no evidence to presume an encephalitis. Steroid effect could have been an additive factor in the mental state.

DR. REINHARD: I would like to present then as the final diagnosis, acute fulminating viral hepatitis. The renal lesion has been thoroughly discussed; the weight of evidence favors hypokalemic nephropathy. I agree with Dr. Levy that there is no convincing evidence to suggest anything other than hepatic coma as being responsible for the terminal mental state. Are there any further comments?

DR. SHANK: We have not yet considered certain other important problems faced by the physicians caring for this patient. These should be mentioned.

The patient was admitted with evidence of severe hepatic necrosis. He was in a confused state and there seemed to be hazard of serious bleeding episodes since prothrombin levels were so very low. The procedures utilized in initial therapy included the use of fresh plasma (to afford an extrinsic source of clotting factors), hydrocortisone and Achromycin.® With these procedures there was some improvement in the patient's general condition and prothrombin levels were increased to somewhat higher values. However, with the onset of hypokalemic alkalosis, the steroid originally given was withdrawn and predinisolone was substituted. By the end of the second week, complaint of burning epigastric pain became a common complaint and the patient was delirious and irritable at times. Efforts were made to reduce the dosage of steroids slowly. Unfortunately, this attempt was associated with deepening coma and steroid dosage was again increased.

The physicians attending him thought that the patient had improved significantly during the first two weeks of therapy. However, his course thereafter was unfavorable, despite the fact that liver function tests seemed to indicate no further evidence of advancing hepatic failure. Purpuric spots with a tiny central pustular area appeared on the skin of his chest. Blood cultures yielded no growth, however, and other evidence of superimposed infection did not present itself. Another late event which remained unexplained was the occurrence of marked and sustained tachycardia. The final conclusion of the attending physician was that death resulted from hepatic failure probably accompanied by superimposed staphylococcal infection.

DR. REINHARD: I am impressed with Dr. Shank's comment regarding infection.

DR. SCHATZ: Hemorrhage from the gastrointestinal tract should also be mentioned as a possibility particularly in view of the epigastric pain.

DR. LEVY: If the liver cannot inactivate the steroids at a normal rate, does their administration to patients with severe hepatic insufficiency lead to much higher levels than we might anticipate under normal circumstances?

DR. DAUGHADAY: As far as I know this subject has not been critically studied in acute hepatic disease. Certainly in chronic hepatic disease the inability to reduce the double bond in ring A is markedly impaired and interruption of this first step in steroid reduction should lead to a higher circulating steroid level.

PATHOLOGIC DISCUSSION

DR. W. STANLEY HARTROFT: The case today centers around the liver. I will also describe the kidneys, lungs, heart and the central nervous system in that order. Dr. Victor Rizk performed this autopsy and it is with his agreement that I present the gross findings as well as the microscopic.

The liver, contrary to the finding on the patient's admission to hospital, was not enlarged at autopsy; it weighed only 900 gm. Some irregularities and small nodules were seen on both uncut and cut surfaces. Presumably, therefore, some degree of regeneration had occurred. (Associated were nearly 2 L. of ascitic fluid.) The organ was red and soft and the capsule wrinkled. There is no question that it was considerably smaller than normal with appreciable loss of tissue, despite the small nodules of regeneration.

Microscopically (Fig. 1), small nodules of regenerating parenchyma were widely separated by strands of loosely woven reticulin in which scattered liver cells persisted that were degenerated or necrotic. An inflammatory exudate, predominately mononuclear and lymphocytic, infiltrated the periportal areas. The stroma in these regions of scarring stained only palely by methods used to demonstrate connective tissue, indicating its recent formation. Most of the severest damage and cellular necrosis was, however, clearly non-portal.

In non-necrotic periportal regions regeneration was evidenced by liver plates two cells wide. (Fig. 2.) There was evidence also of the formation of pseudocholangioles and this process also indicated an attempt at regeneration. Reticulum, however, was not destroyed anywhere and persisted in the damaged areas as condensed stroma. Intralobular bile stasis and intracellular accumulation of the same pigment were seen, indicating disorganization of bile excretion by distortion of architecture. Hemorrhage was present in the most recently injured areas in which frank parenchymal necrosis was evidently progressing.

Histologically, the lesion was not that of acute viral hepatitis, but rather that of a subacute progressive hepatitis. It had been active for a somewhat longer period than that from the time of hospital admission to death, suggesting perhaps a more insidious onset than the history suggested.

The kidneys, in contrast to the liver, were large and their combined weight was over 400 gm. They were bile-stained, swollen and congested, particularly at the corticomedullary junction. This appearance made us think of the nephrosis that is frequently seen in patients who die with severe hepatic disease. The glomeruli were unusually cellular and in some there was a frank glomerulitis. (Fig. 3.) Many of the tubules were swollen and their nuclei necrotic. Others contained bile-stained casts. In addition, a vasculitis was present affecting interlobar and afferent arterioles; this lesion was caused by invasion of a fungus, Aspergillus. Juxtaglomerular cells were hypergranulated—a finding often associated with the presence of ascites and disturbed sodium balance.

In the lungs there was a bronchopneumonia in all lobes. Microscopically it appeared to be of

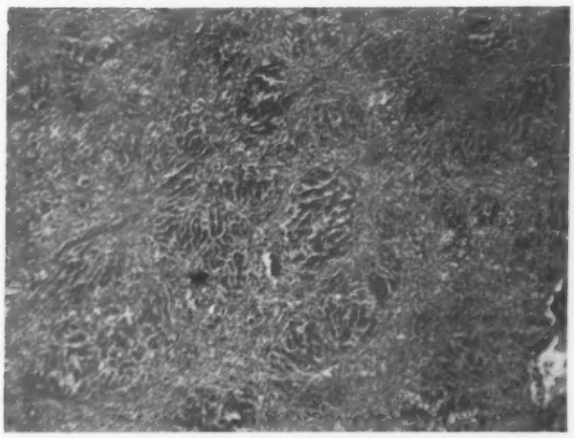

FIG. 1. Low power view of the liver stained with hematoxylin and eosin. Small regenerating nodules of the hepatic tissue are widely separated by strands and bands of connective tissue in some areas of which hemorrhages occurred.

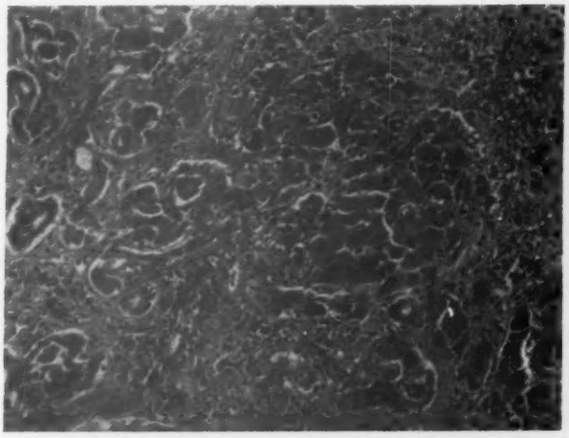

FIG. 2. Nodules of regenerating tissue and pseudocholangioles indicate that the process has gone on for some time and has reached a subacute stage.

several weeks' duration in some areas. (Fig. 4.) Alveolar epithelization had progressed to considerable degree in many of these areas. In the more acutely affected regions, Aspergilli were found in large number.

In the heart scattered foci of necrosis containing many fungi were present throughout. On gross examination they were evident in the form of small yellow areas surrounded by bright red halos. They were found throughout various portions of the myocardium but were rare in the pericardium. (Figs. 5 and 6.)

The brain was large, weighing over 1,400 gm. Areas of hemorrhage and softening were present in every portion including the pons, cerebellum, basal ganglia, midbrain, frontal and occipital lobes. Microscopically, fungi were found in all these softened areas just as in the kidney, lung and heart, and were characterized by polymorphonuclear reaction. In the posterior pituitary a circumscribed area was affected in the same way. (Fig. 7.)

I think the interpretation that was made by one of the clinicians is an excellent one. The picture is that of hepatitis with regeneration. Had the patient survived, this lesion would have progressed to classic Laennec's cirrhosis rather than postnecrotic cirrhosis. The hepatic damage found at autopsy was not sufficient to account solely for the patient's death. The extensive lesions of the kidney, lung, heart and brain due to Aspergillus infection contributed,

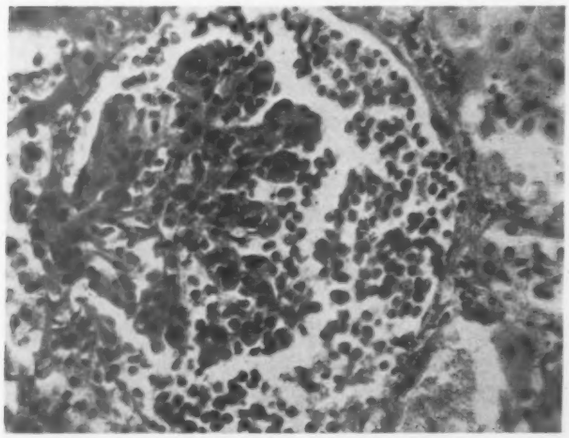

FIG. 3. Polymorphonuclear leukocytes are present in Bowman's space and have infiltrated through the capsule of this glomerulus. The surrounding tubules show degeneration and sudden loss of nuclear staining.

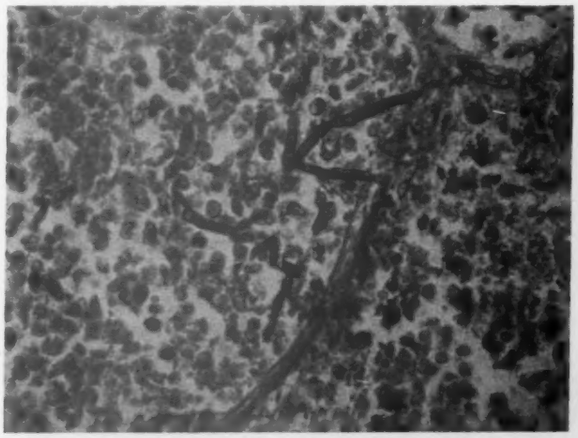

FIG. 4. Branching forms of Aspergillus are seen in the polymorphonuclear exudate filling this alveolar space. High magnification.

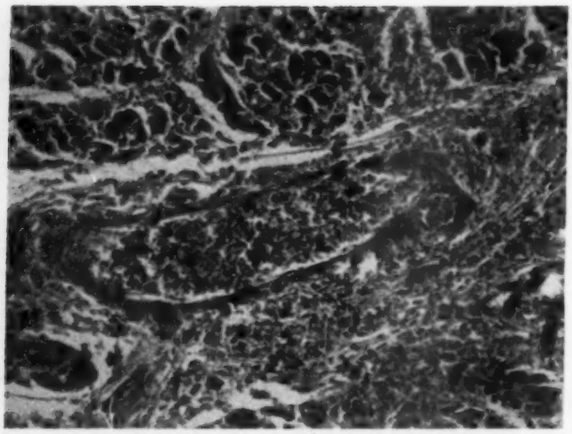

FIG. 5. The branch of the coronary artery shown above is involved in an acute vasculitis and fungal forms (Aspergillus) could be demonstrated in the lumen and penetrating the wall. There is an acute inflammatory reaction in the surrounding tissue.

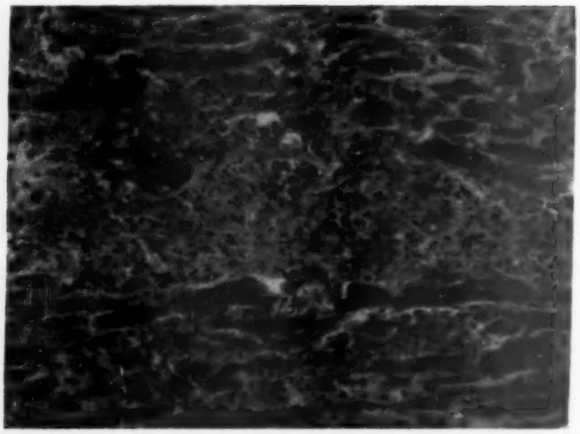

FIG. 6. Small abscesses had formed throughout the myocardium in many areas such as above. An acute polymorphonuclear exudate characterized tissue reaction with destruction of muscle fibers. Aspergilli were demonstrated in these areas with suitable stains.

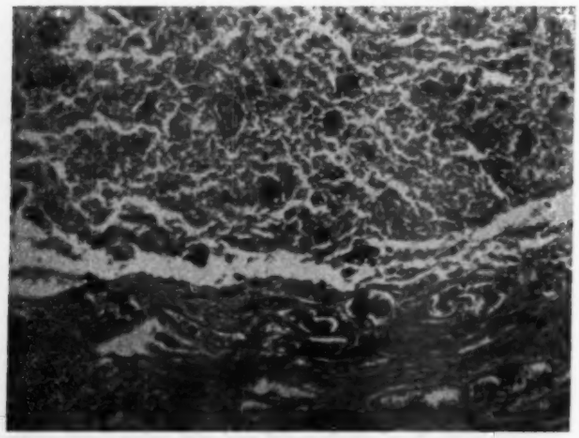

FIG. 7. An area of necrosis is illustrated in this photograph of a section of the posterior pituitary. Similar areas of necrosis were demonstrated by means of serial sections throughout the entire gland. The necrosis is in the upper portion of the picture.

along with the hepatic damage, to his death. The lesion in the posterior pituitary may not have been three weeks old, but it may explain the diuresis. The onset of hypoxic nephrosis may be responsible for the later disappearance of diuresis. The changes in the heart were not old enough to explain the apparent friction rub that was heard at the time of admission. Finally, I would like to refer to the lack of tenderness of this patient's liver at the time of admission. I have difficulty in explaining this lack because three or four weeks before death this liver must have been much more acutely inflamed than it was at the time of death and at the time of admission to the hospital. I would have thought it would have been more enlarged and tender. The lack of tenderness may suggest that that whole process was even older than I have hinted.

DR. SHERRY: Were there any gastrointestinal lesions?

DR. HARTROFT: Yes, throughout there were non-specific erosions, ulcerations (multiple small ulcerations) and hemorrhages of the type so frequently seen in very ill patients. No fungi were demonstrated.

DR. REINHARD: I suspect each one will have to decide for himself whether the posterior pituitary lesion was responsible for the diuresis. There is certainly still a possibility that the patient had hypokalemic nephropathy, which was corrected clinically.

DR. HARTROFT: The final anatomical diagnoses are subacute hepatitis, extensive with nodular regeneration; severe generalized jaundice; acute hypoxic nephrosis with bile casts; ascites (1,700 cc.); degeneration and necrosis of posterior pituitary (history of diuresis one week before death); focal areas of hemorrhagic necrosis of myocardium, kidney and cerebellum containing fungi (Aspergillosis); pericardial effusion (50 cc.); hydrothorax, bilateral, 100 cc. left, 100 cc. right; focal areas of subendocardial hemorrhage; ecchymosis and petechiae along the small intestines; and acute superficial ulcerations in the esophagus and stomach.

Case Reports

Chronic Histoplasmosis*

Report of a Patient Successfully Treated with Amphotericin B

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HISTOPLASMOSIS is now recognized as a common fungus disease occurring over much of the United States, including some areas formerly considered non-endemic [*2*]. In its usual form histoplasmosis exists as a benign and self-limited pulmonary infection. Less commonly, a chronic disseminated and sometimes fatal form of the disease may be encountered, for which no effective therapy has hitherto been available. It is the purpose of this report to present a case of chronic disseminated histoplasmosis successfully treated with amphotericin B (fungizone®).†

CASE REPORT

A. D., a fifty-three year old white male carnival concessionaire, was admitted to the Strong Memorial Hospital on July 15, 1957, with a chief complaint of a tongue ulcer present for four months. The patient had made his home in Rochester, New York, all of his life but for the past twenty-five years had worked as a concession operator in a carnival traveling through the eastern and midwestern parts of the United States. He was first seen in this hospital in November 1947 with symptoms of Buerger's disease, for which a right lumbar sympathectomy was performed. At this time he gave a history of frequent profuse night sweats for the previous two years. His chest was noted to be moderately emphysematous and the fingers and toes displayed marked pulmonary osteoarthropathy. His temperature was normal during his hospital stay. The patient was not seen again until November 1955 when he presented himself for surgical repair of an inguinal hernia. The findings on general physical examination were unchanged from those of eight years before, but the patient again mentioned having occasional night sweats. Postoperatively his temperature spiked to 38 to 38.5°C. orally each night. X-ray examination of the chest showed finely nodular accentuated bronchovascular markings throughout both lung fields, thought to be suggestive of chronic disease of the lung.

The patient was hospitalized elsewhere in March 1956 for surgical excision of a parotid tumor which he had first noted a month previously. Again the history records a complaint of night sweats and fever. Physical examination was as before but for the additional finding of a 3 cm. right parotid mass. The excised tumor microscopically proved to be a papillary cystadenoma lymphomatosum of the parotid gland (Warthin's tumor). In addition, several areas of necrosis and granulomatous inflammation were observed in portions of the lymphoid tissue included in the tumor. (Fig. 1.)

The patient was again hospitalized elsewhere in November 1956 with a complaint of three weeks of hoarseness. Over the previous four to five months he had lost 20 pounds in weight, had become anorexic, and noted some increase in frequency of his night sweats, together with the development of a non-productive chronic cough. There was no history of hemoptysis, chest pain or known exposure to tuberculosis. Physical examination showed a chronically ill patient with evidence of weight loss, but was otherwise unchanged. Routine hemogram and urine were normal but for a corrected erythrocyte sedimentation rate of 29 mm. (Wintrobe). A P.P.D. skin test (second dilution) showed a 2 cm. area of induration at forty-eight hours. The patient's oral temperature rose to 38 to 38.5°C. on several occasions during his first few hospital days. X-rays of the chest showed no change from previous films. Laryngoscopy performed on November 26 was reported as normal. Seven cultures and three guinea pig inoculations of sputum, gastric washings and bronchial washings yielded no tubercle bacilli. One fungus culture of bronchial washings was negative. On December 7 the patient underwent an exploratory left thoracotomy, and 2 mm. nodular lesions were found studding the interlobar and diaphragmatic pleura. Numerous fibrous adhesions were encountered at the apex of the lung.

† The amphotericin B used in this study was supplied through the courtesy of Dr. Gavin Hildick-Smith, Squibb Institute, New Brunswick, New Jersey.

*From the Department of Pathology and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York.

FIG. 1. A portion of the papillary cystadenoma lymphomatosum excised from the right parotid gland. Areas of granulomatous inflammation are present in the upper half of the field. Hematoxylin and eosin. Original magnification, × 50.

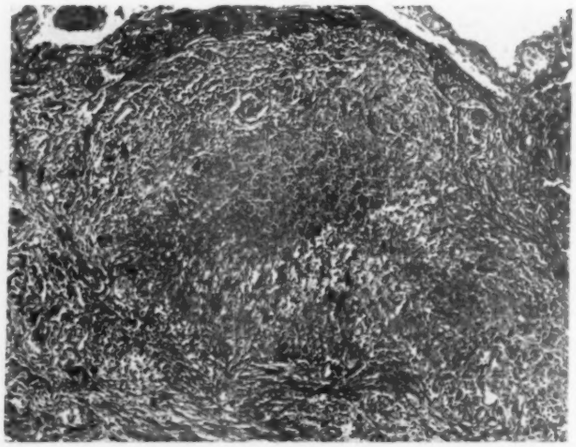

FIG. 2. Biopsy specimen of the right lung showing an area of necrosis and granulomatous inflammation. Hematoxylin and eosin. Original magnification, × 70.

In the parenchyma beneath these adhesions was an irregularly bounded 2 to 3 cm. fibrotic area within which was found a 5 mm. area of necrosis. This area was biopsied and smeared and cultured for tubercle bacilli and fungi. No organisms were identified on smear or culture. Histologic sections revealed chronic granulomatous inflammation with areas of coagulation necrosis and fibrosis. (Fig. 2.) No acid fast bacilli or fungi were identified in the tissue sections at this time.

The patient made an uneventful recovery from surgery and on December 20, 1956, was transferred to a local tuberculosis sanatorium with a presumptive diagnosis of pulmonary tuberculosis. He had lost a total of 34 pounds in weight since March 1956. Hoarseness and sore throat persisted. Laryngoscopy now showed marked ulceration and inflammatory infiltration of the larynx presumed to be tuberculous in origin. Five gastric washings and two sputum specimens were negative for tubercle bacilli on smear and culture. Antituberculosis therapy was started on December 29, 1956, and was continued to May 6, 1957. In this period he received 6.4 gm. of duostreptomycin, 372 gm. of para-aminosalicylic acid and 36.8 gm. of isonicotinic hydrazide. No clinical improvement was observed during this period of therapy. The patient continued to be febrile intermittently, with temperature to 38°C. The larynx failed to heal. On March 13, a small ulcer was first noted on the left anterior edge of the tongue. The patient continued to be anorexic and lost an additional 25 pounds while in the sanatorium. At his request, he was discharged unimproved on May 6, 1957.

The patient's condition gradually deteriorated over the ensuing weeks. His voice was reduced to a whisper. The tongue ulcer enlarged to 1 cm. in diameter and became so painful that he was obliged to subsist on a liquid diet. A biopsy of the tongue ulcer was obtained on June 15, 1957, and submitted to us for pathologic examination. The microscopic section revealed a chronic granulomatous ulcer. (Fig. 3.) Sections stained for acid-fast bacilli revealed no organisms, but sections stained by the periodic acid-Schiff and methenamine silver nitrate methods disclosed large numbers of organisms morphologically consistent with Histoplasma capsulatum. (Fig. 4.) The paraffin blocks of the previous surgical specimens of parotid tumor and lung were obtained and sections from these stained with methenamine silver nitrate. Organisms resembling Histoplasma were identified by us in both tissues.

Because the patient was obviously deteriorating physically and psychologically, he was admitted to the Strong Memorial Hospital on July 15, 1957. His total weight loss now amounted to 54 pounds. He was a chronically ill, wasted-appearing, white male in constant pain from his throat. His voice was a hoarse whisper. The tongue ulcer biopsy site showed no evidence of healing. The pharynx was severely injected. The chest was emphysematous. Marked pulmonary osteoarthropathy persisted in the digits. Laryngoscopy revealed edema, ulceration and inflammatory exudate involving most of the larynx. The right vocal cord was fixed. Admission laboratory work showed a hematocrit of 37 per cent, hemoglobin of 13.6 gm. per 100 ml.; urine specimens showed a specific gravity of 1.008 to 1.014, no albumin or sugar, and 4 to 10 red blood cells per high power field in the centrifuged sediment. A chest x-ray revealed slight accentuation of the diffuse fibrosis of both lung fields in comparison with the films taken two years earlier. An old tuberculin skin test 1:1000 showed a 0.5 cm. area of induration at forty-eight hours; a parallel histoplasmin skin test showed a 3 cm. area of induration and erythema. Coccidioidin and blastomycin skin tests were negative. Serological studies were obtained, as tabulated in Table I. A Congo red test was negative. The blood Wassermann test was negative. The

FIG. 3. Biopsy of the edge of the ulcer of the tongue showing extensive granulomatous inflammation. Hematoxylin and eosin. Original magnification, × 30.

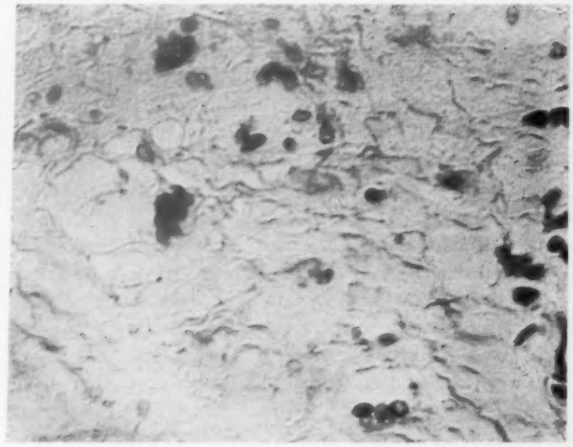

FIG. 4. High power view of the base of the ulcer of the tongue shown in Figure 3. Organisms morphologically identified as H. capsulatum are present singly and in clumps. Methenamine-silver nitrate. Original magnification, × 970.

baseline blood urea nitrogen was 19 mg. per 100 ml., urea clearance 20 per cent and 22 per cent. The ulcer was cultured once and the urine twice for fungi, without success. Gastric washings, unfortunately, were not obtained.

Therapy with intravenous amphotericin B was begun on July 21 in an initial dose of 0.25 mg. per kg. of body weight, diluted in 1,000 cc. of 5 per cent glucose in water. This was administered as a slow intravenous infusion over a period of four to five hours. Daily intravenous treatments were given with the dose of amphotericin B increased to 0.50 mg. per kg. over the first week. Daily infusions were continued until September 1, at which time they were interrupted because of recrudescence of symptoms suggestive of a peptic ulcer. A gastrointestinal series revealed a deformity on the lesser curvature of the stomach. Laparotomy on September 3 by Dr. E. B. Mahoney disclosed a thoracoabdominal aortic aneurysm and no gross abnormality of the stomach or duodenum. No hepatomegaly was observed but the spleen was estimated to be approximately four times normal size. Enlarged mesenteric and retroperitoneal lymph nodes were also noted.

Treatment with amphotericin B was reinstituted on September 11 and continued to October 13. In all, the patient received sixty-eight doses of amphotericin B for a total of 1,640 mg. of the drug over a two and a half month period. He also received orally 2 gm. of gantrisin® daily for nineteen days during the period from July 24 to August 12.

Marked subjective and objective improvement in the patient's condition occurred during the period of therapy. Within a month of onset of treatment all pain had disappeared from the tongue and throat. The tongue and laryngeal ulcers had healed and the patient was eating solid food again. Intermittent ulcer-type epigastric pain, however, persisted.

The patient was followed up with biweekly white blood counts, weekly urea clearance determinations, and several bromsulphalein® tests, for evidence of drug

TABLE I

RESULTS OF SEROLOGICAL STUDIES FOR EVIDENCE OF HISTOPLASMOSIS IN THE PATIENT PRESENTED*

Date	Complement Fixation Titer with Yeast-like Cell Antigen of H. Capsulatum	Histoplasmin Titer	Comments
7/12/57	125	115	Patient clinically ill
8/1/57	210	175	Ten days after beginning of therapy
10/30/57	90	130	Lesions completely healed; seventeen days after cessation of treatment
11/27/57	45	55	No clinical evidence of histoplasmosis remaining
12/27/57	30	60	Patient clinically well

NOTE: These tests were carried out under the supervision of Dr. Elizabeth Hazen, Associate Bacteriologist, New York State Department of Health, Division of Laboratories and Research, Albany, N. Y.

* Parallel studies with blastomyces antigen were negative in each instance.

toxicity. All white counts were in the range of 5,000 to 12,000 per cu. mm. The urea clearances during the first six weeks of treatment were all in the range of 20 to 30 per cent. By September 17 the urea clearance had risen to 51 per cent and by November 19, five weeks after the conclusion of therapy, a 79 per cent urea clearance was demonstrated. The bromsulphalein tests all gave normal results.

An immediate systemic reaction to the amphotericin B was present early in the course of the patient's therapy. This was manifested by a severe shaking chill occurring initially one to two hours after the beginning of the infusion and lasting twenty to thirty minutes. Associated temperature spikes as high as 40°C. were observed. After therapy had been in progress for about ten days the chill was delayed until after the end of the infusion and finally, after three weeks of treatment, failed to materialize. It was found that the oral administration of 0.650 gm. of sodium acetylsalicylate at the beginning of the infusion, repeated at the first symptom of a chill, frequently aborted the reaction to the drug.

Although the patient apparently developed tolerance to the pyrogenic factors in the first lot of amphotericin used, he again reacted severely when treatment was continued with a second lot of the drug despite no lapse in the treatment schedule. Tolerance to the second lot of amphotericin then developed. This was lost when therapy was interrupted for ten days and was subsequently re-established.

The patient has now been discharged from the hospital and has received no medication for three months. He is afebrile, and subjectively and objectively free from evidence of active histoplasmosis.

COMMENTS

Amphotericin B is an antifungal agent offering much promise for the treatment of deep mycoses. It is a derivative of an unnamed species of streptomyces and is produced in forms for oral and intravenous use [*1,4*]. The drug is insoluble in water and hence intravenous administration of the suspension must be carried out slowly and with caution. In its present form, the drug is chemically irritating and tends to produce a phlebitis in the veins used for administration, but no serious complications otherwise seem to attend its use.

Rubin et al. [*3*] have recently reported success in the treatment with intravenous amphotericin B of a case of acute histoplasmosis with x-ray evidence of pulmonary infiltration. The authors comment, however, that "the prognosis of acute pulmonary histoplasmosis that is not clinically disseminated to other body organs is almost invariably good without any specific therapy. Therefore, evaluation of 'specific' therapy must be interpreted with caution."

In our case a more certain correlation can be made between the clinical improvement of the patient and the therapeutic exhibition of amphotericin B. Although positive cultural evidence was not obtained, the clinical picture, biopsy evidence with morphologic demonstration of the fungus, and high antibody titers serve to confirm the diagnosis of histoplasmosis in this case beyond reasonable doubt. The positive histoplasmin skin test, of course, indicates only past or present infection. The serological evidence, however, is of much greater significance. As Rubin et al. have stated, "The demonstration of a rising or falling antibody titer in association with or after clinical symptoms is considered presumptive evidence of infection with H. capsulatum. Serologic tests are positive (1:8 or greater) only during the acute stages of the disease, and the titer falls when healing occurs. The higher the titer in a single specimen, the greater is the probability of active disease." [*3*]. The fall in titer from previously very high levels with clinical improvement under therapy is considered highly significant in this case.

In view of the therapeutic results in this patient, further clinical trial of amphotericin B in the treatment of histoplasmosis appears to be indicated.

SUMMARY

1. A case is presented of chronic histoplasmosis involving parotid gland, larynx, tongue, lung and probably kidneys, spleen and abdominal lymph nodes successfully treated with a new antifungal agent, amphotericin B.

2. Further clinical trial of amphotericin B in the treatment of histoplasmosis is indicated.

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Idiopathic Insulin-Resistant Diabetes Mellitus*

Report of a Case Associated with Insulin Allergy

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EVER since insulin has been used in the treatment of diabetes mellitus, the failure of certain patients with diabetes to respond in the expected manner to apparently physiologic substitution therapy has aroused the curiosity of many investigators. Whereas high insulin requirements have been observed under a number of conditions complicating the clinical picture of diabetes, cases of true insulin resistance have been rather uncommon. Root [*1*] defined a case of insulin-resistant diabetes as one requiring 200 or more units of insulin daily for more than forty-eight hours, in the absence of acidosis and infection. About sixty such cases have been reported in the world literature. We have studied a case of insulin resistance and insulin allergy over a period of four years. Certain aspects of this case pertaining to the use of tolbutamide have been previously published elsewhere [*2*].

CASE REPORT

A fifty-six year old white man was first noted to have diabetes mellitus in May 1951. He was given a diabetic diet of 1,800 calories and 20 units of NPH insulin daily. His insulin requirements could be gradually reduced and by January 1952 he was well controlled by diet alone. He did well until December 1953 when his diabetes became worse. He was given 40 units of NPH insulin and 25 units of crystalline insulin daily. Despite a gradual increase of his daily insulin dose to 120 units, the blood sugar on his first admission to the hospital in March 1954 was 400 mg. per 100 ml. and he showed 4-plus glycosuria and 2-plus ketonuria. Physical examination was essentially within normal limits except for the presence of multiple, firm, non-tender, movable, subcutaneous nodules in the abdominal wall which the patient stated were the sites of previous insulin injections.

Laboratory data other than those pertaining to carbohydrate metabolism revealed no abnormalities. Urinary 17-ketosteroid excretion was normal. A roentgenogram of the chest was within normal limits.

There was no evidence of any infection. Despite a gradual increase of the daily insulin dose to 400 units, glycosuria and ketonuria persisted and the patient lapsed into severe acidotic coma on the ninth hospital day. Only after 9,500 units of crystalline insulin had been given within the ensuing three days did the acidosis subside. For the next three weeks his daily insulin requirements ranged from 1,800 to 6,000 units of crystalline insulin. A mild upper respiratory infection and a small area of cellulitis observed during this time may have been partly responsible for the patient's enormous insulin needs. However, after all symptoms and signs of an infection had subsided the daily insulin requirements still remained high. Figure 1 illustrates the average daily insulin dose as calculated for each week of hospitalization.

During the sixth and seventh weeks of hospitalization three doses of nitrogen mustard, 7 mg. each, were administered in an attempt to depress the formation of insulin antibodies. A marked drop in the daily insulin requirement was subsequently noted, followed by a resurgence about five weeks later. (Fig. 1.) During the sixteenth week of hospitalization administration of 80 units of ACTH gel daily was started and continued for ten weeks. During this time the daily insulin dose was reduced from 750 units to 250 units, and finally remained fairly stable at 125 units. (Fig. 1.)

In addition to his insulin resistance the patient showed signs and symptoms of insulin allergy, manifested by local reactions at the site of injection as well as generalized urticaria following intramuscular and intravenous injections of insulin. Intradermal injection of insulin and passive transfer tests confirmed the presence of insulin allergy. A biopsy specimen of the liver revealed no evidence of cirrhosis or hemochromatosis. The patient was discharged from the hospital in January 1955. He was then receiving a diabetic diet of 3,000 calories and 75 units of Lente insulin plus 50 units of crystalline insulin before breakfast.

* From the Veterans Administration Center, Togus, Maine.

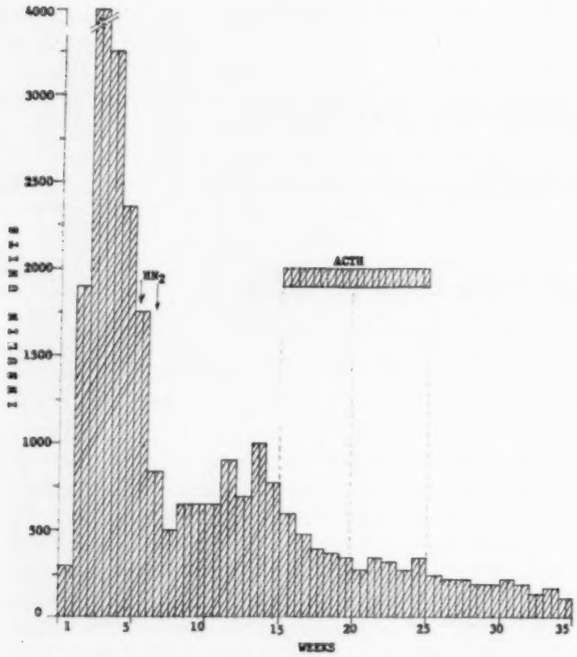

FIG. 1. Average daily insulin dose calculated on a weekly basis during hospitalization in 1954.

He was readmitted to this hospital in August 1956, after his daily insulin requirements had again climbed to about 1,000 units a day and ketonuria had reappeared. With appropriate insulin dosage the ketonuria subsided promptly, but very large amounts of insulin were necessary to keep the blood sugar and glycosuria reasonably well controlled. No evidence of any other underlying disease could be found. The urinary excretion of 17-ketosteroids and glucocorticoids was within normal limits.

The daily insulin dosage varied greatly from day to day and on one occasion was as high as 4,100 units. Only crystalline insulin (500 units or 100 units per ml.) was used. Following administration of tolbutamide* the daily insulin requirements gradually dropped to 475 units. After discontinuance of tolbutamide the daily insulin dose rose again to 900 units. In order to eliminate any delay in insulin absorption all insulin was given intravenously. The patient's diabetes was well controlled when the entire daily dose was administered in one intravenous injection at 7:30 A.M. This response was attributable to the marked retardation of the hypoglycemic effect of the intravenously given insulin and its slow dissociation from the insulin-binding factors in the serum, as shown later on. This therapeutic regimen was no longer feasible when the insulin-binding power decreased in the course of the treatment. After the daily insulin dose of 900 units had remained stable for over two weeks, tolbutamide was resumed and again a

* Kindly supplied as orinase® by the Upjohn Company, Kalamazoo, Michigan.

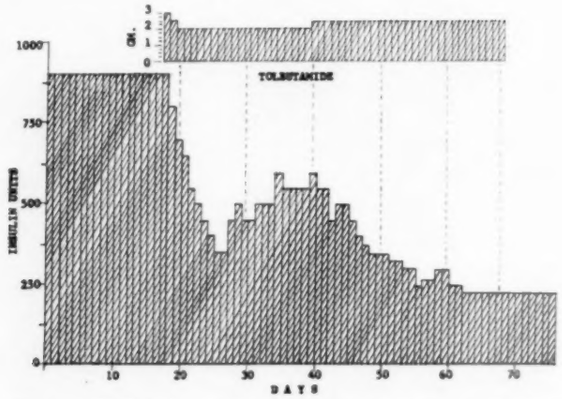

FIG. 2. Daily insulin requirement before and after tolbutamide.

marked drop in the daily insulin requirement was observed. Figure 2 illustrates the prompt reduction of the daily insulin dose following tolbutamide administration [2]. Upon discharge from the hospital in May 1957 the patient required 150 units of crystalline insulin daily. When he was readmitted in December 1957, tolbutamide was again given in a dose of 2.5 gm. daily. Within ten days the daily insulin dose could be reduced from 165 units to 110 units and has remained stable since.

COMMENTS

In the absence of diabetic acidosis, infection, hormonal overactivity or any other condition known to cause abnormally high insulin requirements, our case can be classified as one of idiopathic insulin resistance associated with insulin allergy.

Skin-sensitizing antibodies, present in insulin allergy, were demonstrated by a strong local reaction following the intradermal injection of 0.02 units of crystalline insulin. The passive transfer test for these antibodies performed according to Prausnitz-Küstner was also strongly positive.

The simultaneous occurrence of resistance and allergy to insulin has been reported in a number of cases. However, the association between these two conditions is by no means constant. In their review of the pertinent literature Davidson and Eddleman [3] found only seventeen cases of insulin allergy among fifty cases of insulin resistance. On the other hand, insulin allergy without resistance has been observed in 7.3 per cent of diabetic patients receiving insulin [4], Lowell [5] proved the existence of two different antibodies to crystalline insulin, one being responsible for skin sensitivity, generalized urticaria and other allergic manifestations, the

other causing insulin resistance. With the help of zone electrophoresis of the serum the skin-sensitizing antibody has been localized in the beta-globulin fraction [*6*] whereas the insulin-neutralizing antibody has been found to be associated with the gamma globulin fraction [*7*], specifically with the gamma-2-globulin fraction [*8,9*].

These factors inhibit the hypoglycemic effect of exogenous insulin and have been variously described as insulin antibodies, inhibitors and binding factors. Studying the effect of serum from insulin-resistant patients on the combination of insulin with the rat diaphragm preparation, Marsh and Haugaard [*10*] found that the "binding factor" had to be present at the same time the diaphragm was exposed to insulin in order to exert its antagonistic action. In contrast to their observation, Field, Stetten and Woodson [*11*] proved the presence of a different insulin antagonist in the serum of patients in diabetic acidosis that was rapidly and firmly bound to the diaphragm independent of insulin and then interacted with insulin to abolish its effect. This factor had an electrophoretic mobility similar to the alpha-globulins and was not present in the gamma globulin fraction. Thus these authors have shown that inhibition of insulin activity during diabetic acidosis is fundamentally different from that seen in idiopathic insulin resistance.

Insulin-binding factors have been found in all insulin-treated subjects and only in those [*12*]. Berson and co-workers [*13*] found that in insulin-treated patients insulin-I^{131} was bound to an acquired globulin which satisfied the criteria for an antibody and which they called "insulin-transporting antibody." Its appearance was observed after three to four months of insulin medication. According to these authors the maximal capacity for insulin-binding in non-resistant subjects rarely exceeded 10 units of insulin per liter of plasma, whereas it amounted to 80 to 400 units per liter of plasma in four insulin-resistant patients [*14*]. The insulin-binding antibody retains the insulin, which normally disappears from the blood quite rapidly, much longer and prevents it from entering the cell. Studies with I^{131}-labeled insulin have demonstrated that in insulin-treated persons 15 per cent to 90 per cent of injected insulin is still present in the blood one hour after injection, whereas only about 8 per cent is found in persons who have not been previously treated with insulin. Normal degradation of insulin is similarly delayed [*15*]. Thus the binding factors interfere with the biological activity of insulin, which depends upon the quantity of the hormone available at the cellular level and not upon its concentration in the blood. When these factors are present in excessive quantity, insulin resistance results.

Why such large amounts are produced in some cases and under certain circumstances is still obscure. Insulin itself is a weak antigen, but impurities and additions to prolong its action may give rise to the initial formation of insulin antibodies [*16,17*]. A similar phenomenon was observed by Gordon et al. [*18*] following injection of other hormones. They found that purified hormone preparations were less antigenic than crude ones. These investigators concluded that true immunological reactions played a role in the production of antihormones following injection of hormone preparations containing antigenic material. Immunizations or infection may also raise the titer of insulin antibodies in the form of an anamnestic reaction. Rausch-Stroomann [*16*] found an increased insulin-antibody titer fifteen to sixty minutes after injection of a large insulin dose. Further insulin administration may thus serve as a booster, especially if it is given intermittently or had been discontinued for some time. This was observed in many of the published cases of insulin resistance and was considered an important factor in the development of resistance by some authors [*9,19*]. In the case herein described insulin therapy was interrupted for about one year, after 20 units of NPH insulin had been given initially for eight months. We concur in the assumption of other investigators [*7,15,19–21*] that insulin resistance is a true immunological phenomenon.

Our case showed what others have observed, namely, localization of the insulin-binding antibodies in the gamma globulin fraction of the serum. This was accomplished by starch block electrophoresis and addition of insulin-I^{131}. An interesting quantitative relationship between the insulin-binding power of the gamma globulin fraction of our patient's serum and the severity of his insulin resistance could be established. At a time when 98 per cent of the added insulin-I^{131} was bound to gamma globulin, the daily insulin dose was 800 units. When only 42 per cent of insulin-I^{131} was attached to the gamma globulin fraction, the daily insulin requirement had

TABLE I
PLASMA BINDING OF INSULIN-I^{131}*

Sample	Binding of Insulin-I^{131} to Gamma Globulins (%)	Daily Insulin Dose (units)
Patient's serum 9-26-56.....	98	800
Patient's serum 1-18-57.....	42	270
Serum of non-insulin-resistant diabetic patients.....	<6	20–60

* Starch block electrophoresis; insulin concentration 0.2 units per ml.

TABLE II
INSULIN SENSITIVITY TEST*

Sample	Blood Sugar (mg./100 ml.)		
	Before	30 Minutes Later	60 Minutes Later
Before tolbutamide.	240	240	235
After tolbutamide for 9 days.....	240	230	220
After tolbutamide for 50 days.....	170	130	65

* Blood sugar response to intravenous injection of 100 units of crystalline insulin.

dropped to 270 units. (Table I.) The oft-described ability of the serum of insulin-resistant patients to protect mice from the hypoglycemic action of insulin was also demonstrated in our patient. A sample obtained when the daily insulin requirement was 800 units was capable of protecting mice from the lethal effects of 0.07 units of insulin. Only one of seven mice died when given that amount of insulin and 1 ml. of the patient's serum, while forty-one of forty-nine animals died when given the same amount of insulin and 1 ml. of normal serum.

While the aforementioned tests and also the measurement of insulin activity by the rat diaphragm technic [*10,22*] require elaborate laboratory facilities, insulin sensitivity tests and blood sugar profiles afford a simple method of detecting abnormal inhibition and retardation of insulin activity. In our case we have found blood sugar profiles extremely helpful for the demonstration of insulin-binding factors as well as for the management of the diabetes. Perusal of the literature reveals that this diagnostic tool has been rarely used in cases of insulin-resistant diabetes. By this clinical method one can often arrive at a more accurate estimate of insulin-resistance than by the rat diaphragm technic, insulin-I^{131}-binding method or the mouse protection test. Since all these tests are based on the presence of free binding factors, their saturation by previously administered insulin may yield falsely negative results in highly insulin-resistant patients [*3,11,18*]. Some investigators were unable to demonstrate the insulin-neutralizing activity of the serum in about 50 per cent of insulin-resistant patients [*23*].

Collens and Banowitch [*24*] considered the insulin sensitivity test more important for the diagnosis of resistance than the actual amount of insulin required. Insulin sensitivity tests, performed during different phases of our patient's clinical course, reflected the immense insulin resistance and its decrease after treatment with tolbutamide. The test dose of insulin used (1.5 units per kg.) was of necessity fifteen times as large as the usual dose of 0.1 unit per kg. (Table II.) No tolbutamide was given during the twenty hours preceding the tests.

On several occasions the blood sugar was determined about every two hours throughout the day while the patient remained on his regular meal schedule and received his usual insulin injections. Two blood sugar profiles obtained before tolbutamide medication, when the daily insulin doses were 1,000 units and 1,200 units, respectively, reveal the amazing inhibition or retardation (or both) of the hypoglycemic effect of large amounts of insulin intravenously given. Even after intravenous injection of 1,200 units of crystalline insulin (500 units per ml.) the blood sugar reached its lowest level only as late as eight hours after the injection with two meals consumed by the patient in the interval. Following the evening meal the blood sugar was still maintained below the initial morning value. (Fig. 3A.) In contrast to this marked retardation of insulin activity two blood sugar profiles obtained during the second course of tolbutamide administration, when the daily insulin dose had dropped to 400 units of insulin and less, showed considerably less delay of the hypoglycemic effect of the insulin administered intravenously. In both curves the lowest blood sugar level was

reached as early as four hours after the intravenous injection of much smaller amounts of insulin, namely 400 units and 220 units, respectively. (Fig. 3B.)

Inasmuch as only that amount of insulin which is not prevented from entering the cell by insulin-binding factors can exert its biologic effect, the management of insulin resistant diabetes should aim at providing enough free insulin for satisfactory control of the diabetic state. Therapeutic measures should be directed at the following points: (1) saturation of the insulin-binding factors by large, preferably intravenously given, insulin doses, (2) avoidance of further stimulation of insulin-antibody formation by the exclusive use of crystalline insulin, and (3) inhibition of or interference with the formation of insulin antibodies.

Dituri [*25*] found the insulin-antibody titer highest in the morning and lowest in the evening. It has been our experience and also that of others [*26*] that it is therefore important to administer a large insulin dose in the morning in order to saturate the insulin-binding factors. This large dose is usually well tolerated due to the rapid binding of the injected insulin. Its subsequent slow dissociation from the antibody complex often effects satisfactory control of the diabetes throughout the entire day. By giving the insulin intravenously one can avoid its often unpredictable absorption from the tissues due to additional blocking of the insulin by binding factors present in the tissue fluid. However, when, in the course of treatment the increasingly prompt effect of the intravenously administered insulin does not permit large enough amounts to be given in this way, one can take advantage of this additional delay of insulin activity and mimic the effect of depot insulin by giving large amounts of crystalline insulin subcutaneously or intramuscularly. This circumstance made the use of long-acting insulins in our case unnecessary. We believe that in cases of insulin resistance only crystalline insulin should be given as it is less apt to stimulate antibody formation than long-acting insulin preparations which contain more antigenic material.

Alleviation of insulin resistance by suppression of insulin-antibody formation has been attempted by several means. Some investigators [*7,18,21,24,27,28*] saw good results following the use of ACTH or adrenocortical steroids, with complete remission in some cases, while others [*6,9*] noticed no improvement. According to

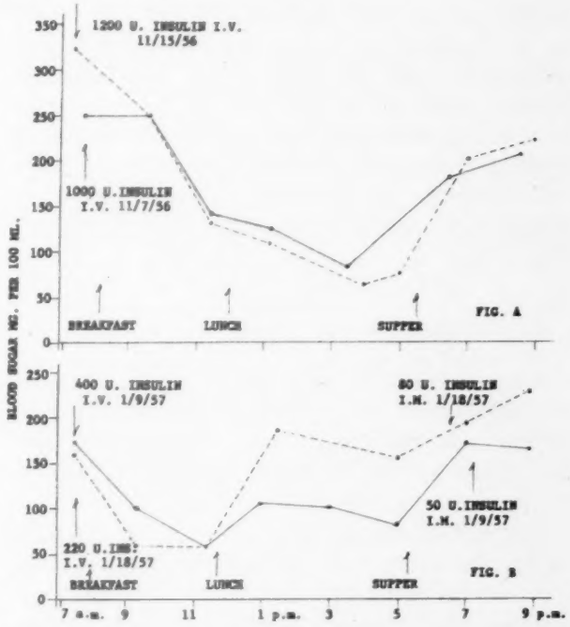

FIG. 3. Blood sugar profiles without (A) and with tolbutamide (B).

Colwell and Weiger [*7*] ACTH and adrenocortical steroids probably interfere with the excessive formation of gamma globulin and block the anamnestic production of specific insulin antibody. Dammin and Bukantz [*29*] reported depression of antibody production in rabbits by the use of nitrogen mustard and Geller et al. [*30*] reduced the insulin requirement of their insulin-resistant patient 90 per cent by the injection of 12.4 mg. nitrogen mustard on two consecutive days. In our patient a marked decrease of the average daily insulin dose was noted following three injections of 7 mg. nitrogen mustard in April 1954. (Fig. 1.) A spontaneous remission cannot be excluded, however, since another therapeutic trial during a relapse of the patient's insulin resistance in June 1956 was entirely unsuccessful. As can be seen from Figure 1, the response to ACTH was quite satisfactory in that the average daily insulin dose could be reduced to 34 per cent of the initial value during a ten-week course of ACTH, and after withdrawal of the hormone dropped further to 16 per cent of the amount required before ACTH was started. The most decisive improvement of insulin resistance was achieved by the use of tolbutamide (orinase). During the past eighteen months our patient received three courses of tolbutamide without any untoward effect. On the first two occasions the daily

insulin requirement decreased 75 per cent, on the third occasion 33 per cent. (Fig. 2.) It has been previously proposed that the insulin-sparing effect of tolbutamide in this case has been due to its inhibitory action on the insulin-binding factors [*2*]. This assumption has been confirmed by the diminished plasma binding of insulin-I^{131} following tolbutamide administration. (Table 1.)

SUMMARY AND CONCLUSIONS

A patient with initially mild diabetes mellitus is described in whom after discontinuance of NPH insulin for a year, severe insulin resistance and moderate insulin allergy developed following resumption of treatment with NPH insulin.

The several distinct plasma factors responsible for insulin allergy, idiopathic insulin resistance, and insulin resistance associated with diabetic acidosis are discussed. Idiopathic insulin resistance is regarded as a true immunological phenomenon.

A good correlation between the plasma binding of insulin-I^{131} and the severity of insulin resistance is shown. Blood sugar profiles are considered a valuable diagnostic tool for the evaluation and satisfactory control of insulin resistant diabetes.

The management of insulin resistant diabetes mellitus is outlined and the effects of nitrogen mustard, ACTH and tolbutamide (orinase), as observed in the treatment of the case reported are described.

Acknowledgment: We are greatly indebted to Dr. James B. Field at the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland, for the determination of the insulin-I^{131}-binding power of our patient's serum and the mouse protection test.

ADDENDUM

Since this paper was written the patient's daily insulin requirements rose again to 400 units. This rise could not be reversed by the administration of tolbutamide. The patient was then given prednisone for four and a half months. The first drop in the daily insulin requirement was observed on the tenth day of prednisone administration. From then on the daily insulin dose could be gradually reduced to 50 units. After discontinuance of prednisone the diabetes has remained well controlled with 50 to 60 units of insulin a day.

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Radiation Nephritis*

Report of a Fatal Case

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DURING the past half century therapeutic radiation has attained an important position in the physician's armamentarium for the treatment of neoplasms. Much has been learned regarding radiation effect on tumor tissue and the many side-effects involving skin, hematopoietic, gonadal, gastrointestinal and pulmonary tissue. However, despite some experimental and more recently clinical evidence to the contrary, there has been widespread belief that the kidney is relatively resistant to radiation. The following case report, we believe, illustrates the extent to which x-rays are capable of inducing inflammatory and degenerative changes in the kidney, termed "radiation nephritis."

CASE REPORT

E. M., SMH No. 399885. This fourteen year old boy was first seen on July 11, 1955, because of painless enlargement of the right testicle noted nine days before. The previous medical history was non-contributory. Physical examination was normal, except for a non-tender, firm, testicular mass 7 by 6 by 4 cm. The blood pressure was 104/68 mm. Hg. Laboratory studies revealed a normal hemogram, negative (sterile) urine, normal serum non-protein nitrogen, and a normal chest roentgenogram and intravenous pyelogram. (Fig. 1.)

On July 19, 1955, radical excision of the right testicle was performed. The tumor removed was a teratocarcinoma. On July 26, 1955, a right, radical, retroperitoneal lymph gland dissection was carried out. No evidence of metastatic tumor was found. On August 8, 1955, a course of deep x-ray therapy to the abdomen was started. Radical treatment through anterior and posterior 10 by 20 cm. ports was carried out on each side of the abdomen. The duration of treatment was twenty-six days, including twenty treatment days, and the total dose employed was 4,400 r maximum and 4,000 r minimum tumor dose. Therapy was well tolerated, although rather marked erythema and some desquamation of the skin developed over each of the treated sites.

The patient did well until December, 1955 when increasing fatiguability developed as well as exertional dyspnea, palpitations and marked pallor, nausea, vomiting and tarry stools. On admission to the hospital on December 28, 1955, examination revealed a markedly pale and asthenic boy. The blood pressure was 145/65 mm. Hg. Positive findings included small left axillary and supraclavicular lymph nodes, and a small left retinal hemorrhage. The heart was enlarged to the left and a loud precordial systolic murmur was heard, but there were no diastolic murmurs. The skin over the anterior and posterior abdomen bilaterally showed moderate x-ray pigmentation and numerous small telangiectases. The remainder of the examination was within normal limits.

There was a profound normochromic anemia with a hematocrit of 11.5 per cent, reticulocytosis of 12 per cent and a white blood cell count of 11,700 per cu. mm. Urinalysis revealed 3-plus proteinuria, microscopic hematuria and pyuria, and occasional hyaline and granular casts. Stool specimens were tarry and gave a 4-plus guaiac reaction. The blood urea nitrogen was 54 mg. per 100 ml.

The patient's symptoms abated following multiple transfusions but no source for the gastrointestinal bleeding was found. Sternal bone marrow aspiration revealed myeloerythroid hyperplasia. No evidence of tumor invasion or x-ray reaction was noted. Intravenous pyelograms revealed poor concentrating ability and failure to visualize drainage structures on the right.

Following discharge from the hospital, uremia and melena persisted which necessitated two further hospitalizations during the ensuing sixty days. The physical findings were unchanged except for the development of labile hypertension and more prominent dermal telangiectases. The second hospitalization in February 1956 was marked initially by increasing azotemia and acidosis. Meticorten® was given in doses of 30 mg. per day in an effort to decrease gastrointestinal bleeding thought to be related to

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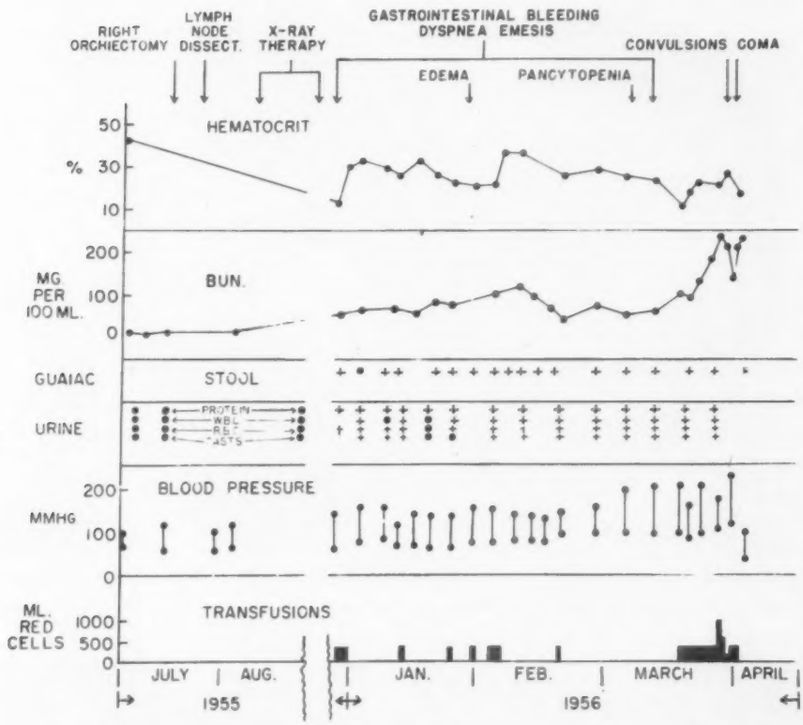

FIG. 1. The main clinical features and laboratory findings are illustrated.

diffuse telangiectasia. The blood urea nitrogen rose to 117 mg. per cent. Two days later progressive improvement occurred associated with sudden return of appetite, considerable diuresis, progressive fall of the blood urea nitrogen to 42 mg. per cent and less gastrointestinal blood loss.

During the following month anasarca developed associated with a fixed hypertension and the administration of meticorten was discontinued. In March 1956, moderate leukopenia and thrombocytopenia were noted for the first time, and there was a question of splenic enlargement. The reticulocyte count averaged 6 per cent. Gastrointestinal bleeding increased and the patient was admitted for the final hospitalization on March 19. The course was progressively downhill. Alimentation and replacement of gastrointestinal blood loss became ever-increasing problems. The leukopenia and thrombocytopenia subsided within a week. The sternal bone marrow findings were unchanged. Uremia became progressively more severe and there was severe hyperchloremic acidosis and hypocalcemia. Convulsions, recurrent tetany and coma finally terminated in death on April 4, 1956.

At autopsy, the left kidney weighed 150 gm., the right 100 gm. On the left, the kidney was firmly bound to the retroperitoneal structures by a dense mass of uniform, gray, fibrous tissue and was removed from the retroperitoneal position with some difficulty. The capsule was adherent, and the underlying surface smooth and pale but distinguished by the presence of numerous punctuate red dots, apparently representing hemorrhagic or congested glomeruli. Cut sections were pale, yellow-gray, with a 4 to 5 mm. cortex in which striations were poorly seen. The glomeruli were again prominent as punctate red dots, some of which were slightly larger and appeared to be petechial hemorrhages. The pyramids were pale but otherwise normal. The arcuate arteries did not appear unduly prominent. The calyces, pelvis and ureter were normal.

The right kidney resembled the left but was more markedly affected. The retroperitoneal fibrosis was particularly marked so that sharp dissection was necessary to remove the kidney from its bed. A 4 to 5 mm. capsule of tough, dense, scar tissue surrounded the kidney, and was very adherent. (Fig. 2A.) The surface resembled that on the left. Cut sections were pale and distinguished by the large number of congested or hemorrhagic glomeruli and by the relative inconspicuous nature of the remaining architecture. The drainage structures were normal.

Microscopic sections from both the right and left kidneys showed the same types of change but these were appreciably more pronounced on the right. (Figs. 2B and C.) The capsules showed a marked degree of hyaline scar tissue thickening. No normal glomeruli remained. Most were scarred, fibrotic and atrophic, with areas of fibrinoid changes within the tufts. Many glomeruli were severely congested or hemorrhagic. The tubules throughout showed atrophy

FIG. 2A. External surface of the bisected right kidney. Note the marked thickening of the partially stripped capsule.

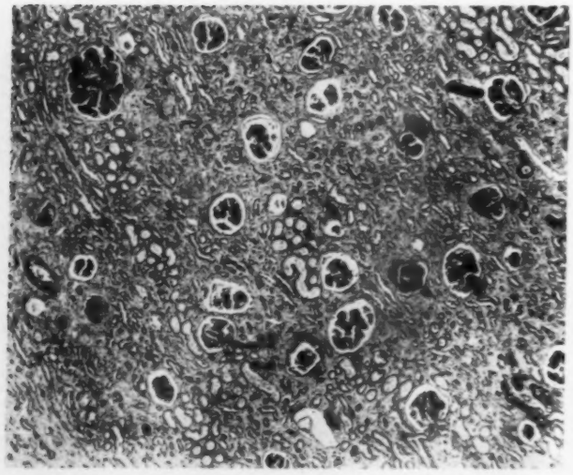

FIG. 2B. Low power view of the right kidney showing extensive glomerular change, interstitial fibrosis, and tubular atrophy. Hematoxylin and eosin.

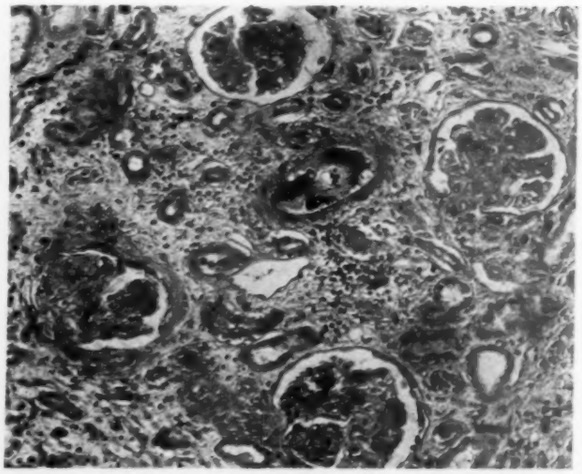

FIG. 2C. Higher power view of the right kidney. Note the glomerular alterations and the fibrinoid change in the walls of the arteriole in the upper center. Phloxine-methylene blue.

of the lining epithelium, some containing hyaline casts. There was marked increase in interstitial scar tissue, moderately heavily infiltrated with mononuclear cells. All the arterioles showed a bluish staining, smudged appearance of the walls, which were thickened to the point of obliteration of the lumens in many instances. The intima of the larger arteries showed patchy similar changes plus marked intimal proliferation and re-duplication of the internal elastic laminae.

Lymphoid tissue was, in general, atrophic. No evidence of tumor was found. The lumbar and sternal marrow cavities were gray-yellow, firm and fibrous appearing. The former was markedly hypocellular, with only a few tiny nests of normoblasts, blast forms, and a rare megakaryocyte scattered through abundant fat. Sections of the sternal marrow were moderately cellular, with large numbers of early forms and normoblasts. Maturing myeloid elements were decreased in number. Megakaryocytes were numerous.

The spleen appeared only slightly enlarged, weighing 270 gm. Normal architecture was maintained. Microscopically, normoblasts and occasional megakaryocytes were seen as well as large numbers of hemosiderin-filled macrophages.

In the gastrointestinal tract blood filled the distal half of the small bowel and the entire large bowel.

Seven cm. distal to the cecum there was a single 7 to 8 cm. shallow punched-out ulceration of the mucosa of the colon. There was injection and slight dilation of small blood vessels beneath the mucosal surface in the area of the cecum and a suggestive similar change in the remainder of the colon. Sections of the ileum and colon showed large numbers of moderately dilated, thin walled venules and capillaries present in the submucosa and lamina propria.

COMMENTS

The subject of radiation nephritis has been extensively reviewed elsewhere [*1*] from both the clinical and experimental aspects. Early investigations utilizing dogs, cats and rabbits, and a variety of x-ray technics radiating both *in situ* and exteriorized kidneys, failed to produce notable renal changes [*1–5*]. In 1919 Hall and Whipple [*3*], using heavy x-ray doses delivered to four thoracoabdominal areas in the dog, failed to produce pathological evidence of chronic renal damage. It would appear from later investigations that these observations, made over a period of three to sixty-two days postradiation, were too early to span the variable latent period now known to occur in chronic radiation nephritis [*4*].

In 1926 the report of Hartman, Bolliger and Doub [*5*] established nephritis secondary to radiation as a pathological and experimental clinical entity. Although some of their dogs died from acute radiation sickness and others from suppurative nephritis or intestinal obstruction, several survived for periods greater than three months. In the latter group a clinical syndrome of chronic progressive renal insufficiency evolved. This was initially associated with albuminuria, cylindrinuria, fixed specific gravity, decreased phenosulfonphthalein excretion and polyuria, and was followed by progressive debility, urea retention, acidosis, hyperphosphatemia, hypertension and death [*6*]. The kidneys of these animals showed a thickened, tense and adherent capsule, increased interstitial tissue and distorted, flattened, atrophic or desquamated tubular epithelium. The glomeruli were well preserved, although the tufts showed variable hyalinization and atrophy. In addition, intimal thickening to the point of complete endarteritis was described and the progressive nature of the lesion emphasized.

During the following year, these studies were confirmed in the dog by Willis and Bachem [*7*] who used radiation to the kidneys directly through an abdominal incision. Between 1930 and 1932 the observations of Bolliger and Laidley [*4*], Bolliger and Earlam [*8*] and Earlam and Bolliger [*9,10*] placed experimental radiation nephropathy on a semiquantitative basis. In 1933 similar renal changes and chronic renal insufficiency without hypertension were produced by the intravenous injection of active deposits of radium [*11*]. In 1936 Page [*12*] was able to develop a picture of chronic renal insufficiency in the dog by direct radiation of each kidney exteriorized to the loin by use of four to eight erythema doses over a six to twenty-one week period. The clinical and pathological features in these animals closely resembled those previously described, although no capsular thickening was noted.

The case report of Dean and Abels [*13*] in 1944 called attention to the development of hypertension following unilateral abdominal radiation. At nephrectomy seven and one-half years later, the kidney in the radiated area appeared contracted in its lower third and replaced by fatty tissue and fibrous septums in which atrophy and marked sclerosis, especially of the blood vessels, was present. The upper half of the kidney appeared normal. Following nephrectomy, the signs and symptoms of hypertension subsided.

In 1950 Zuelzer and co-workers [*14*] established radiation nephritis as a clinical entity. These investigators observed three young children who died in renal insufficiency five to seven months after receiving heavy abdominal x-ray radiation. Similar case reports were presented by Grossman [*1*] and by Smith and Williams [*15*] in 1955. In general, these patients presented similar clinical features and pathological findings. There were few capsular adhesions and little thickening. However, microscopically the glomeruli showed various stages of fibrinoid degeneration and hyalinization, and there was associated tubular atrophy and necrosis, interstitial fibrosis with little inflammation reaction, and variable endarteritis of the arterioles and larger vessels.

The most extensive clinical experience with radiation nephritis in the adult is that of Luxton [*16*] and Kunkler et al. [*17*] reported in 1952 and 1953. These authors described twenty-seven cases of renal disease believed to have developed following abdominal x-ray radiation employed in the treatment of seminoma of the testicle. These patients received approximately 3,000 r (depth) over a five week period, the area of

treatment encompassing both renal beds. Following a latent period of six to twelve months, in thirteen of these patients, five of whom died, evidence of relatively acute onset of moderate or marked renal insufficiency and hypertension developed. The surviving patients, although stabilized, showed evidence of chronic renal insufficiency. Chronic renal insufficiency without acute onset was thought to have developed in eight additional patients, and in six other patients benign or malignant hypertension, presumed to be of similar etiology, was noted. Pathological study of four of these cases revealed renal changes similar in many respects to those previously described both on an experimental and a clinical basis.

Another case report in 1956 [*18*] brought the total number of cases described in the adult to forty-nine, although many of the early reports were not well documented [*6*].

The current case report, we believe, is another example of postradiation nephritis. Against a background of documented good health, this patient received at least 4,000 r (depth) to each side of the abdomen through ports which undoubtedly included both renal beds. The latent period, before onset of symptoms of renal insufficiency, was slightly shorter than noted experimentally and in previous clinical studies. The onset of extensive gastrointestinal bleeding probably brought the renal problem to medical attention earlier than would otherwise have been the case. The period of renal insufficiency, marked by persistent azotemia, low fixed urinary specific gravity, microscopic hematuria, pyuria and cylindruria, and the development of progressive hypertension, closely resembles the clinical features of the disease previously described. Finally, at autopsy the findings of a thickened renal capsule, degenerative glomerular changes, tubular atrophy and interstitial fibrosis and endarteritis duplicate those of previous reports.

The asymmetric variation in the degree of involvement of the two kidneys requires further explanation. It would appear that the more extensive capsular and parenchymal involvement on the right might have been related to the extensive surgery and resultant fibrosis. However, conceivably, the degree of capsular thickening and possibly parenchymal compression noted on the right might have induced a hypertensive picture similar to that described experimentally by Page [*20*] as a result of perinephritic compression. This hypothesis seems less likely, however, when one considers that renal insufficiency was well established before hypertension became a notable feature of the clinical picture.

Other clinical features in this case deserve further comment. The telangiectases over the abdominal wall and back developed quite soon after radiation and were rather extensve. This finding aroused suspicion that gastrointestinal telangiectasis might have been a contributing cause of the protracted melena; the presence of telangiectasis was later confirmed at autopsy. Whether the protracted blood loss hastened the patient's renal failure or even caused deterioration in what otherwise might have been a relatively stable situation remains problematic. Meticorten was employed in the hope of decreasing the degree of capillary bleeding. Although initially, clinical and hematological improvement coincided with its use, it would be difficult to establish a cause and effect relationship. Later in the patient's illness, the steroid appeared to have no appreciable effect, nor did it appear to contribute to the progressive renal insufficiency.

The development of leukopenia and thrombocytopenia near the termination of the illness remains unexplained. This appeared to be transient in nature and of moderate degree. The extramedullary hematopoiesis probably reflected the extreme and protracted stress to which the patient's erythropietic tissues were subjected.

A review of the pertinent literature indicates that nephritis is an unusual complication of radiation therapy. The reasons for its occurrence are not clear. However, there is some clinical evidence to suggest that renal insufficiency may develop only when both renal areas have been radiated with at least 2,500 r (depth) and when less than one-third of the total renal tissue is outside the critical radiation field [*17*]. Others have speculated on the possibility of unusual sensitivity of the young, growing kidney to radiation to explain the development of nephritis during infancy. In any event, the possibility of radiation nephritis should be seriously taken into account in planning abdominal x-ray therapy.

SUMMARY

A case of progressive and ultimately fatal renal insufficiency following abdominal x-ray radiation is described and the pertinent literature on the subject briefly reviewed.

The natural history and prognosis of a given

neoplasm, and the probable efficacy of radiation therapy, should be weighed against the danger of delivering bilateral renal radiation of potentially lethal degree.

Acknowledgment. We wish to express our sincere thanks to Dr. Lawrence E. Young, Professor of Medicine, for his encouragement and helpful criticism, and to the Departments of Radiology and Urology for their assistance in the preparation of the manuscript.

ADDENDUM

Since preparation of this paper a report of three additional surviving cases has been presented by Cogan and Ritter [*21*].

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A Case of Diffuse Histiocytosis X with Aplastic Anemia*

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Cases of diffuse histiocytosis X have not infrequently been reported associated with anemia or pancytopenia. These findings have developed secondary to "toxic" marrow depression [*1*] or replacement [*1–3*], or to hemorrhage [*4–7*], or to autoimmune mechanisms [*5*]. We have been unable to find any previously reported cases of bone marrow aplasia responsible for this hematologic picture. The following report is of a case which presented clinically as a presumed "toxic" aplastic anemia following chloramphenicol therapy for recurrent infections. Postmortem examination demonstrated a diffuse histiocytosis X with bone marrow aplasia.

CASE REPORT

L. H. (OSUH 612733), a six year old white boy, was admitted to the University Hospital on March 26, 1957.

In May 1956, the patient had had the sudden onset of stiff neck, temperature of 106°F. and cervical adenopathy. Because of previous known allergy to penicillin and gastrointestinal intolerance of oxytetracycline, he was given chloramphenicol with complete remission of symptoms. He had a similar episode in July and again chloramphenicol was effective in the suppression of his symptoms. In November his family noticed a beginning change in personality and activity. He became irritable, moody, easily fatigued, complained of generalized malaise and had at least one episode of spontaneous epistaxis. These symptoms continued in varying degrees of severity until January 1957, when a spiking temperature and abdominal pain again developed. The fever subsided after chloramphenicol was administered and it was decided to admit the patient to his local hospital for tonsillectomy. A blood cell count at the hospital revealed a leukopenia with anemia. The patient was thought to have an iron deficiency anemia and was started on oral iron therapy and ascorbic acid. He was discharged without tonsillectomy but two days later purpura developed and he was rehospitalized. He was noted to have a thrombocytopenia. Bone marrow aspiration was performed which was reported as showing an "aplastic anemia." The patient received a transfusion of 250 cc. of whole blood and was started on prednisone, 20 mg. per day. His white blood cell count increased from 2,300 to 12,000 cells per cu. mm. He was discharged, but again readmitted after one week with anemia, leukopenia, and fever. The prednisone was continued and tetracycline, 500 mg. per day, was added to his regimen. He received a transfusion and then was referred to the University Hospital for further treatment. At the time of his admission his chief complaints were anorexia, an 8 pound weight loss, and fever with daily spikes to 106°F.

Physical examination on admission revealed a temperature of 106°F., pulse rate 148, respirations 32 and blood pressure 118/82 mm. Hg. The patient appeared severely and acutely ill. There was a fresh subscleral hemorrhage of the right eye, peridontal oozing of blood from the upper jaw and fresh bleeding from a shallow ulcer of the lower lip. He had non-tender shotty cervical adenopathy. There was diffuse muscular pain to pressure. The liver and spleen were not palpable.

Laboratory data revealed 5 mg. per cent protein in the urine with 1 to 3 white blood cells and 2 to 4 red blood cells per high power field in a centrifuged specimen. The prothrombin concentration was 51 per cent of normal, total protein was 7.5 gm. per cent with 4.3 gm. per cent albumin and 3.2 gm. per cent globulin. The serum bilirubin, thymol turbidity, blood sugar, blood urea nitrogen, cephalin flocculation test and serological relation for syphilis showed no abnormalities. A throat culture grew hemolytic Staphylococcus aureus, coagulase-positive. The blood count revealed 1,500 white blood cells per cu. mm. with a supravital differential of 8 per cent neutrophils, 4 per cent monocytes and 74 per cent lymphocytes. There were 3,280,000 red blood cells per cu mm., 3,820 platelets (Dameshek technic), 11.7 gm. per cent hemoglobin and a hematocrit of 37 per cent. The corrected sedimentation rate was 52 mm. per hour (Wintrobe method). An x-ray of the chest showed

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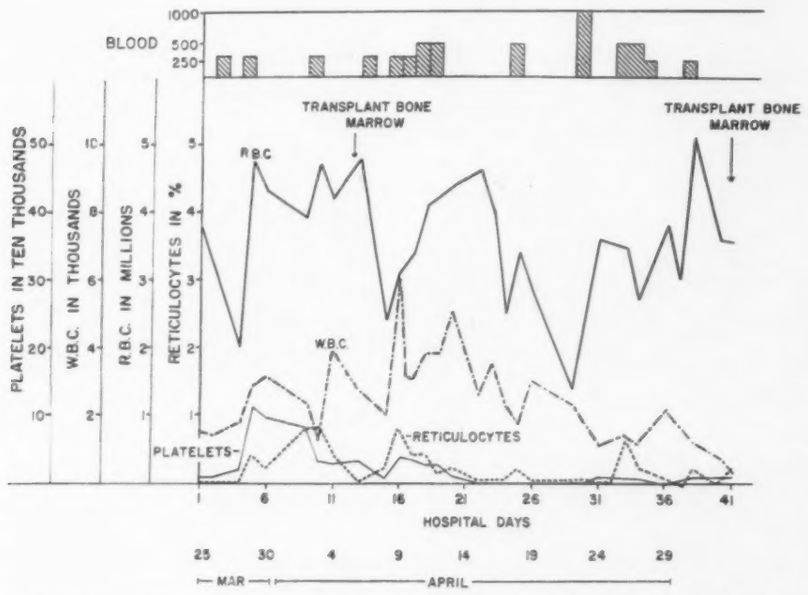

FIG. 1. Graph of hospital course showing effects of bone marrow transplants

slight elevation of the right diaphragm and accentuated markings in the right lower lobe.

Bone marrow aspiration was performed on March 25 in which several fragments of marrow were obtained from the right iliac crest. There was increased fat, clasmatocytes, tissue basophils and fibrous tissue. There were very few granulocytes, and erythroblasts were diminished. There were increased plasma cells and normal-appearing lymphocytes. No abnormal cells were seen. No megakaryocytes were seen. The findings were interpreted as a toxic, damaged marrow with aplasia.

The patient was started on a regimen of prednisone, 20 mg. per day, and erythromycin, 1 gm. per day. He became afebrile after five days but his hemoglobin and red cell count continued to fall. He received several transfusions of fresh whole blood collected in plastic bags in an attempt to relieve the thrombocytopenia and anemia. He continued to do poorly and on April 5 an infusion of bone marrow was given to the patient in an attempt to stimulate normal repopulation of the deficient marrow. This was done by using the patient's mother, who had the same blood type, as the source of the bone marrow transplant. Both the patient and mother were given a high dosage of steroid therapy three days prior to the transfusion. The bone marrow cavity of the hip and an intravenous route were used as the recipient sites. Approximately 2.35×10^8 nucleated cells were delivered. The details of the technic were as has been reported by Thomas et al. [*35*]. Bone marrow examination of the mother was normal while that taken prior to infusion of the patient showed small tissue fragments with increased fat. The erythroid series was almost non-existent and all granulopoiesis was markedly depleted. Only one megakaryocyte was seen. There thus appeared to be continued aplasia of the marrow with no evidence of regeneration. The patient withstood the procedure quite well and no evidence of pulmonary embolism or foreign protein shock was noted. However, as can be seen by Figure 1, there was no evidence of regeneration in the peripheral blood counts.

On the thirteenth day generalized aches developed with sharp pain in the chest and abdominal pain in the right lower quadrant. A flat plate of the abdomen revealed gas-filled segments of small bowel and ascending colon but it was not considered diagnostic of any pattern. The patient started to have temperature spikes to 104°F. every day and stool specimens became consistently positive for occult blood and remained so until his death. He also had an occasional episode of hematemesis. Blood cultures and stool cultures at this time failed to show any pathogenic organisms. On the twenty-first hospital day a maculopapular rash of the face, scalp and trunk developed which was thought to be a drug eruption. This cleared three days after discontinuing the antibiotics. The patient's condition continued to deteriorate. He was given intravenous hydrocortisone and tetracycline and was maintained on intravenous fluids and daily infusions of fresh whole blood.

Another bone marrow transplant was accomplished, this time using his sister who was the same compatible blood type, as the donor. Again the same technic was used as previously described. During this procedure samples of both his sister's and his bone marrow were obtained. The former appeared normal but that of the patient showed a continued aplasia with a preponderance of lymphocytes, clasmatocytes, monocytes, plasma cells and tissue basophils. Almost

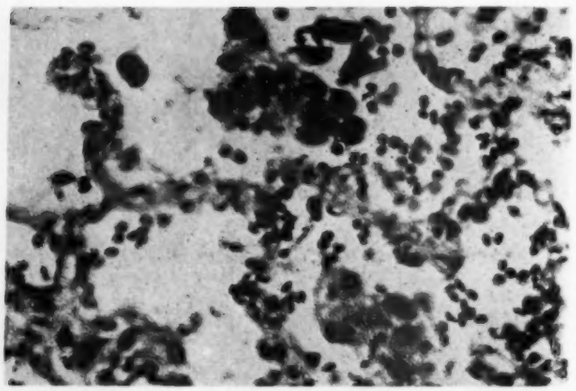

FIG. 2. Section of lung showing collections of foam cells in alveolar spaces.

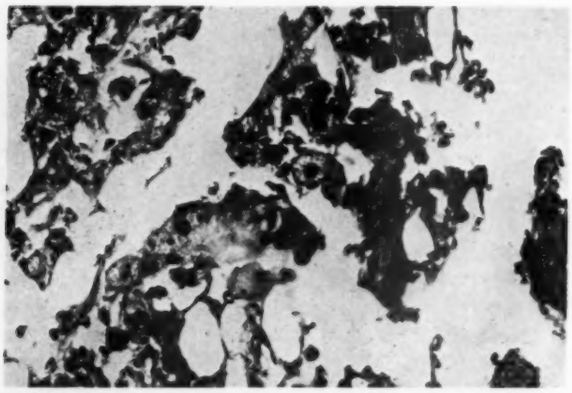

FIG. 4. Postmortem bone marrow showing definite hypoplasia with numerous lymphocytes, plasma cells, histiocytes and several large foam cells. Note absence of myelophthesis.

no granulocytes or red cell elements were seen. No abnormal cells were noted in this totally fatty marrow. There was no evidence of marrow regeneration following the initial bone marrow transplant.

He withstood the procedure quite well but again no evidence of regeneration was noted. (Fig. 1.) Approximately three days after this procedure massive hematemesis, tarry stools and gross hematuria developed, and he died on the fortieth hospital day.

Postmortem examination revealed the following: Grossly, the skin showed jaundice, petechiae and ecchymoses. There was moderate lymphadenopathy in the cervical, axillary and inguinal region. The liver weighed 750 gm. and was 2 cm. below the right costal border. The cut surface of the liver showed numerous large, yellow, firm masses with central necrosis. Culture of these masses grew klebsiella. The spleen weighed 750 gm.

Microscopically, the lungs showed intrapulmonary hemorrhage and infiltration with large foam cells. (Fig. 2.) The gastrointestinal tract showed numerous septic ulcerations. The liver, in addition to the abscesses, contained swollen, foamy Kupffer cells and diffuse reticuloendothelial hyperplasia. The spleen and lymph nodes were infiltrated with large groups of

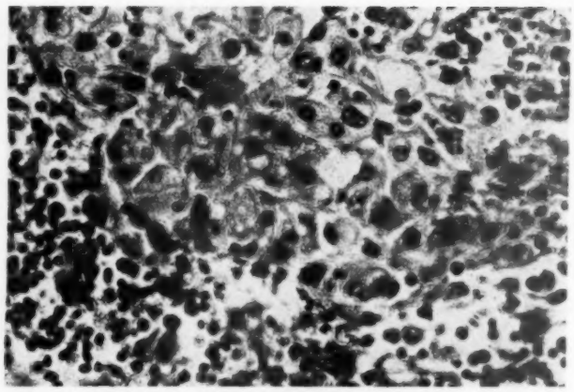

FIG. 3. Section of spleen showing normal architecture in lower left corner with a nest of foam cells in upper center.

reticuloendothelial foam cells. (Fig. 3.) The bone marrow showed toxic necrosis, fatty infiltration and occasional groups of large foam cells. (Fig. 4.) A postmortem blood culture was also positive for klebsiella.

Comment: This patient meets almost all the criteria necessary for the diagnosis of Letterer-Siwe disease (diffuse histiocytosis X) as reported by Varga et al. [*8*] in their review of this problem. The patient had marked splenomegaly, moderate hepatomegaly, hemorrhagic tendency, generalized non-tender lymphadenopathy, anemia, leukopenia and fever [*9*]. The pathological specimens revealed a diffuse hyperplasia of the reticuloendothelial cells. The only criteria missing were localized bone tumors.

In the available literature covering the non-lipoid reticuloendothelioses there is much confusion as to terminology and the boundaries delineating eosinophilic granuloma, Letterer-Siwe disease and Hand-Schüller-Christian disease. Classically, an eosinophilic granuloma is considered to be a benign, localized bone lesion [*10*]. Hand-Schüller-Christian disease is a chronic, systemic disease occasionally giving the triad of exophthalmus, diabetes insipidus and punched-out lesions of the skull. Letterer-Siwe disease is usually an acute, systemic disorder of infants which rapidly and invariably leads to death [*8,9,11*]. Some cases have appeared in the literature in which the disease is described in adults [*12–14*]. However, there are reports of patients progressing from one to another of these classifications [*15*]. Lichtenstein holds the view that these diseases are different manifestations of one basic disorder, hence he proposed the term "histiocytosis X" to include all forms of this disorder. We believe that this term would fit our case better than either of the older clinical

classifications. The etiology of histiocytosis X is still in doubt [*16*]. Whether it represents a primary proliferation of the reticuloendothelial cells [*17*] or a secondary response to infection [*18*] has been the subject of much speculation and investigation. The role of infections in these diseases was mentioned by Letterer [*19*]. Siwe [*20,21*] cited otitis media as part of the clinical syndrome. Varga et al. [*8*], in their review of the literature, also noted that many of these cases were associated with otitis media and mastoiditis. Lipton [*5*] points out that infections are so frequently associated with reticuloendotheliosis that they may be synergistic to a more basic mechanism. One wonders whether these patients might not have decreased resistance to infections because of a functionally inadequate reticuloendothelial system. Recently Wall et al. [*22*] have reported cases of hypogammaglobulinemia associated with reticuloendothelial diseases.

Several authors have recently reported good response of these diseases to antibiotics, especially chloramphenicol. Cox [*23*] and Bierman et al. [*24*] were the first to show that combinations of antibiotics were quite effective. Our patient responded early in the course of his disease to chloramphenicol. Again one wonders whether the effect of these antibiotics might have been on the etiologic agent itself or on secondary infections which may serve to complicate some primary conditions.

Morton [*25*], Bass [*26*], Blahd [*27*], Mermann [*28*] and Cox [*23*] have reported the use of corticosteroids in several patients with good results, some with complete remission. While prednisone was administered to our patient for its myelostimulative action, there seemed to be no effect upon the basic disease process.

In most of the cases reviewed in the literature the hematological findings were late manifestations of histiocytosis X. The most striking findings are the marked splenomegaly, the hemorrhagic tendency, generalized glandular enlargement and localized tumors of bone. When the hematologic findings have become apparent it is predominantly as a progressive, refractive anemia and occasionally a pancytopenia. Few bone marrow studies have been done antemortem and the usual postmortem findings have been bone marrow hyperplasia or reticuloendothelial cell proliferation with secondary replacement of the normal marrow. Some of the anemias may have been due to autoimmune hemolytic or hypersplenic mechanisms. At other times there has been the picture of non-specific marrow toxicity. No known cases have been reported with a picture of complete marrow aplasia. In our case the hematological condition was the presenting symptom, the only clinical evidence of a reticuloendotheliosis initially being moderate cervical adenopathy and fever. The finding of complete bone marrow aplasia, as in our patient on three antemortem samples, confirmed by a postmortem survey, without heavy reticuloendothelial myelophthesis has not been reported before.

As to the mechanism of the aplastic anemia in this case, we can only speculate whether it was part of a previously unrecognized symptom complex of histiocytosis X or a secondary complication to chloramphenicol therapy. Since Volini et al. [*29*] first called attention to the hemopoietic changes occurring with chloramphenicol therapy over 1,700 cases have been reported in the literature. More than 600 of these were aplastic anemia. According to the classification of Lewis et al. [*30*], this patient would fit into his second group, i.e., chloramphenicol was given along with other drugs, chemicals or x-ray. This group totaled 149 patients at the time of his survey. It is noteworthy that the literature since 1954 has contained few reports of chloramphenicol toxicity. During this period several workers have attempted to show the relationship between chloramphenicol and aplastic anemia. In experiments with Rhesus monkeys in these laboratories, Saslaw, Doan and Schafer [*31*] were unable to produce aplastic anemia after either prolonged or intermittent administration in animals with a normal or previously damaged marrow. In more recent experiments it has been demonstrated in dogs that a combination of dietary deficiency plus high doses of chloramphenicol or other antibiotics produces changes in the peripheral blood and bone marrow similar to those seen in aplastic anemias [*32*]. Thus, there are many factors inherent in the mechanisms of the production of bone marrow depression. Wallington et al. [*33*], reporting on a five-year experience with chloramphenicol in 2,100 patients, "found no hematopoietic depression attributable to the drug."

Since the pendulum has seemed to swing away from implicating chloramphenicol as the etiological agent in all cases of aplastic anemia associated with its use, we feel justified in reporting this case as an instance of aplastic anemia which possibly is a part of the complex histiocytosis X.

SUMMARY

A case of a six year old white boy with diffuse histiocytosis X is presented. Bone marrow transplantation was attempted without success on two occasions. The case is of special interest in that complete bone marrow aplasia was present. The possible relationship of the bone marrow aplasia to chloramphenicol therapy is discussed.

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Pseudoxanthoma Elasticum*

Report of a Case

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PSEUDOXANTHOMA elasticum (PXE)† is a relatively rare connective tissue dystrophy. Patients with this disorder may present themselves to the dermatologist or plastic surgeon with the complaint of wrinkled skin, particularly in the region of the neck. They may first gain medical recognition on ophthalmological consultation because of failing vision. The internist who is acquainted with the diverse manifestations of PXE, particularly the peripheral vascular and gastrointestinal facets, may have the occasion to unveil an undiagnosed case. A patient showing the typical skin changes, the typical fundus changes, and the frequently noted changes in the peripheral pulses is herein described.

CASE REPORT

In L. P., a twenty-seven year old woman, blurred vision developed in the left eye on July 4, 1957, two days after the onset of an acute upper respiratory infection. Because of the persistence of impaired vision she sought medical attention on July 8.

Past history revealed that she had worn glasses since the age of seventeen following an episode of acute tonsillitis at which time there was some diminution in vision in both eyes. The skin changes to be described were first noted in the region of the neck at the age of seven. ("I always wanted mother to make my dresses high around the neck.") She denied symptoms of ulcer and there was no history of gastrointestinal bleeding. Careful inquiry into peripheral vascular symptomatology revealed only that her lower extremities ached after walking about a mile and continued to ache for about an hour after completing such a walk. Other than occasional menstrual irregularity, no abnormal bleeding tendencies had been noted.

On physical examination, the patient appeared to be in good health. The skin of the anterior portion of the neck appeared abnormally wrinkled and lax, considering the age of the patient, and on closer inspection the surface was covered with small flesh-colored papules, resembling somewhat the skin of a plucked chicken. (Figs. 1 and 2.) Both antecubital areas showed slight changes of a similar nature. The skin of the lower part of the abdomen appeared moderately wrinkled and lax, but these findings were difficult to interpret because of striae of previous pregnancies.

Ophthalmological examination showed corrected vision in the right eye to be 20/20, left eye 20/80. Ocular tension was normal in each eye. Funduscopy of the right eye showed an irregular ring of pigmentation surrounding a flat, normal appearing disc. Extending outward from the disc were several typical appearing angioid streaks. The left fundus (Fig. 3) showed angioid streaks and peripapillary pigmentation. Several small scattered areas of hemorrhage and exudate were seen in the immediate vicinity of the macula. The macular reflex was obscured and a faint haze of the vitreous near the macula was evident. A central scotoma was present corresponding in size to the macular lesion.

The only other noteworthy finding on physical examination was the peripheral vascular changes. Both radial pulsations were of decreased amplitude, the right being barely palpable. The right dorsal pedal pulse was absent and the left faintly palpable; both posterior tibial pulses were absent. The brachial and femoral pulses were normally palpable bilaterally. No trophic skin changes were noted in the hands or feet. The blood pressure reading in both arms was 110/70 mm. Hg, in both legs 120/80 mm. Hg. Oscillometric readings with the Collens sphygmo-oscillometer were as follows:

Area	Normal	Right	Left
Below the knee	4 to 10	1	1½
Above the ankle	1 to 5	½	½
Forearm	3 to 10	2½	3½

† Following the lead of McKusick [*1*], PXE will be used in the remainder of this article to designate pseudoxanthoma elasticum.

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FIG. 1. Involvement of the skin in the region of the neck. The white thread-like area on the right side of the neck is artefact.

FIG. 2. The abnormal laxity of the skin is demonstrated.

Complete blood count, urinalysis, sedimentation rate, electrocardiogram and chest x-ray film were within normal limits. A roentgenogram of the right lower extremity showed slight calcification in the course of the deep femoral artery.

A biopsy specimen of skin taken from the neck area confirmed the clinical impression of PXE.

On July 15, 1957 the macular lesions appeared less acute and vision had improved. On September 14, 1957 the macular area showed pigmentation and healing. Corrected vision in the right eye was 20/20, left eye 20/30.

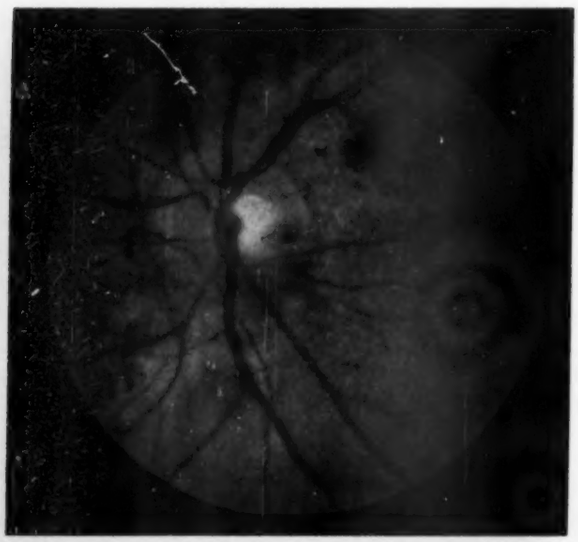

FIG. 3. Photograph of left fundus showing peripapillary pigmentation and hemorrhages in the macular area. Angioid streaks are present but are not too well shown in this photograph.

COMMENT

Pseudoxanthoma elasticum derives its name from the appearance of the skin and the underlying degeneration of the elastic tissue. Microscopic examination of the skin shows a degeneration of the elastic tissue in the deeper layers of the dermis and in these areas the overlying epidermis displays small plaques resembling xanthomas, which on biopsy contain no xanthomatous material but are composed of curled, degenerated fibers with an affinity for elastic tissue stains. These fibers are generally considered to be derived from elastic tissue, but recent investigations indicate the possibility that they are altered collagen tissue, the so-called elastotic degeneration of collagen tissue [*2,3*].

As a result of the underlying connective tissue degeneration the skin becomes lax, furrowed and dotted with pin-head to split-pea size yellowish plaques, symmetrically distributed, and most prominent in the flexural folds of the neck, axillas, extremities and abdominal wall. The appearance of the skin has been likened to that of a plucked chicken [*4*], or coarse grained Moroccan leather [*1*], or orange peel [*1*]. The skin changes may be quite subtle, cases having been described in which the skin appeared normal but biopsy showed the classic changes of PXE. In our case the skin changes

were not marked and might have been attributed to the normal wrinkling seen with aging, except for the relatively young age of our patient.

Angioid streaks of the fundus have been ascribed to tears in Bruch's membrane of the choroid, presumably a degeneration of the elastic tissue in this area [*5–7*]. A dissenting view attributes the streaks to excessively pigmented, affected vessels in the choroid [*8*]. On funduscopy they appear as gray to red-brown pigmented streaks with serrated margins, extending outward from the optic disc in a manner resembling vessels.

The combination of angioid streaks and the skin changes of PXE is known as the Grönblad-Strandberg syndrome, after the ophthalmologist and dermatologist who first correlated these findings in 1929. In 1941 a review of 139 cases of angioid streaks reported since 1929 revealed that the skin changes of PXE were present in 59 per cent, absent in 13 per cent, and not mentioned in 18 per cent [*9*]. Although most commonly associated with PXE, angioid streaks have also been described in osteitis deformans. In a review of sixty-five cases of osteitis deformans, angioid streaks were described in 8 per cent [*9*]. In one case of osteitis deformans, PXE and angioid streaks both were present [*10*]. A recent report mentions a case of osteitis deformans with angioid streaks and occlusive vascular disease, but without manifest skin changes of PXE [*11*].

The visual disturbances, most commonly partial loss of central vision, result from changes in the macular area, usually presenting as macular and perimacular hemorrhages. Proliferative, exudative and atrophic choroidal changes may be seen less commonly.

The connective tissue degeneration also involves the arterial walls, and peripheral vascular manifestations appear. Weak or absent peripheral pulses are found in many of these cases, and symptoms of intermittent claudication may be elicited [*1,11–14*]. Carlborg in his treatise on the circulatory changes in this syndrome attributes the pulse changes to disturbances in the pulse propagation incident to the elastic tissue degeneration of the vessels [*13*]. Obliterative vessel changes have been demonstrated in a few cases on histologic examination [*1,11*]. These findings in a young person should lead one to suspect PXE.

Other vascular manifestations such as aortic aneurysm, intracranial aneurysm, coronary sclerosis with angina pectoris, and an increased incidence of arterial hypertension have been reported. Premature and advanced arteriosclerotic vessel changes is an additional feature in some of these cases. On necropsy in two cases endocardial and pericardial plaques containing degenerated elastic tissue have been described [*1*].

There appears to be a tendency for upper gastrointestinal hemorrhage in these cases, again attributable to vascular changes in these areas [*1,12,14–16*]. X-ray studies usually fail to reveal the source of the bleeding. In one case gastroscopy showed large petechial areas and in another case surgical exploration revealed a friable gastric mucosa with oozing blood from the entire gastric mucosa; unfortunately, no gastric biopsy specimen was obtained [*16*]. In another case a partial gastric resection was performed because of massive upper gastrointestinal hemorrhage in a twenty-three year old woman; histologic study of the resected portion of the stomach revealed elastic tissue degeneration of the gastric arterial vessels with formation of microaneurysms and intimal obliterative sclerosis containing calcified plaques. Three cases of upper gastrointestinal bleeding associated with PXE and pregnancy have been described recently, and the effects of pregnancy on elastic tissue are discussed [*16*].

Nasal, bladder, uterine and subarachnoid hemorrhage have been reported, as have spontaneous hemarthroses and purpura, without disturbance of blood clotting factors [*1*].

Chest roentgenograms in several cases have shown miliary mottling, the nature of which has not been ascertained, although it has been theorized that it may be secondary to interstitial hemorrhages with deposits of hemosiderin [*1,14*]. An increased incidence of neurologic and psychiatric disorders is mentioned as occurring in this syndrome, but the evidence for this is not conclusive. Three cases of diabetes mellitus, three of thyrotoxicosis, and one of diabetes insipidus constitute the flimsy evidence for association of endocrine abnormality with this syndrome [*4*].

PXE is considered to be a hereditable disorder transmitted as an autosomal recessive partially limited to the female, with occasional transmission as an autosomal dominant [*1,17*]. In our case, there are two children, a two year old girl and a six year old boy, neither showing any skin changes suggestive of PXE. Six siblings

of the patient, five of whom are females, were unavailable for examination.

SUMMARY

Pseudoxanthoma elasticum is a connective tissue dystrophy with typical skin changes, fundus appearance, and peripheral vascular manifestations. A typical case displaying this triad is presented. Other manifestations are discussed.

Acknowledgment: The authors are indebted to John M. Knox, M.D., Associate Professor of Dermatology, Baylor College of Medicine, for confirmation of the findings on examination of the biopsy specimen of skin in this case.

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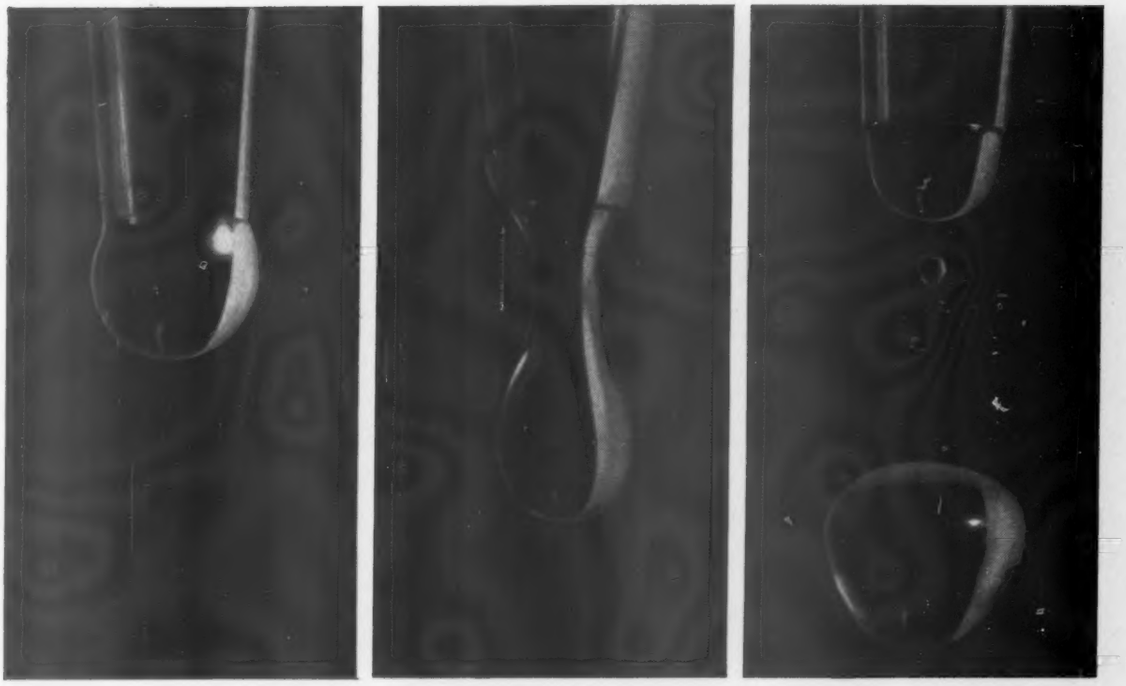

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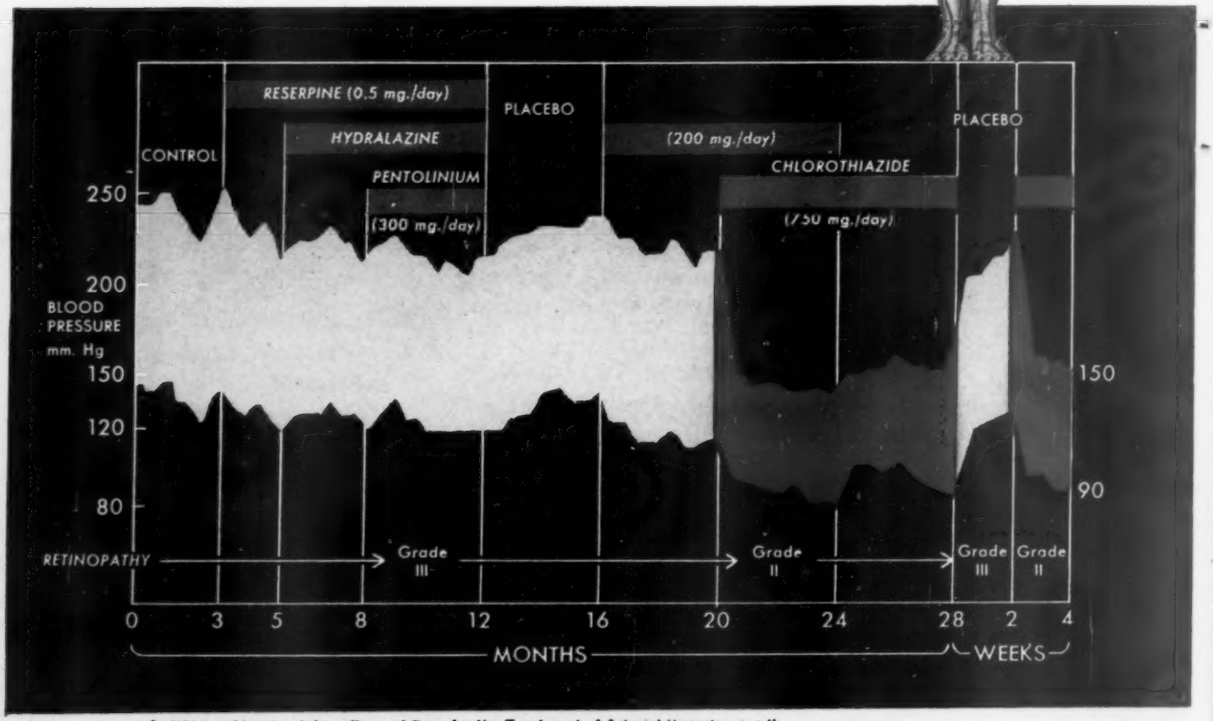

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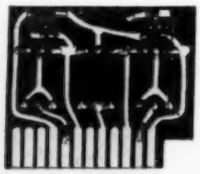

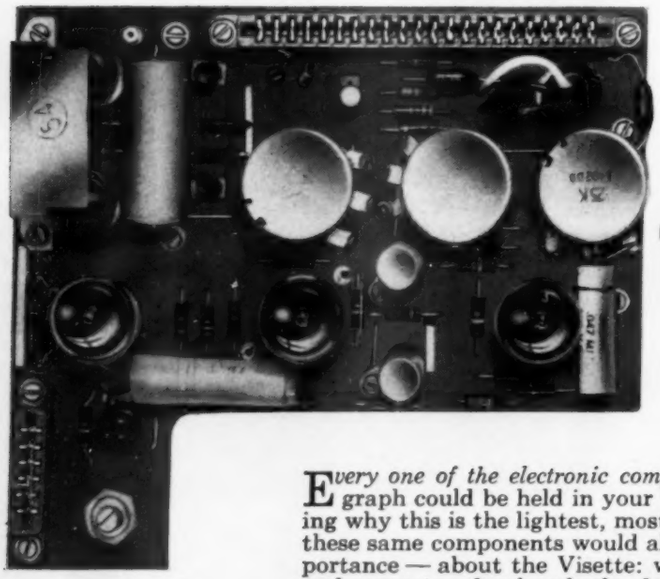

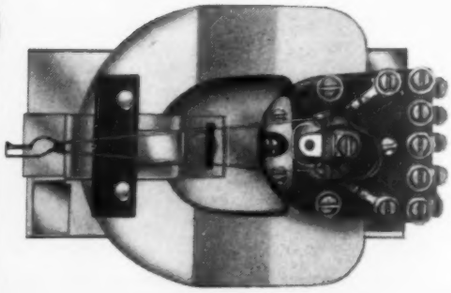

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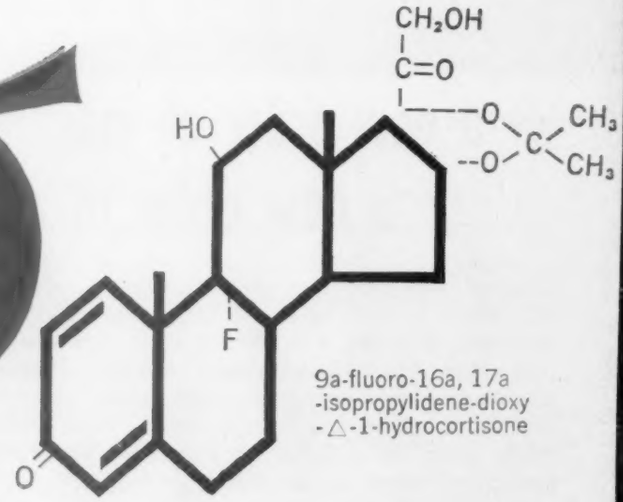

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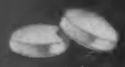

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Northridge, California

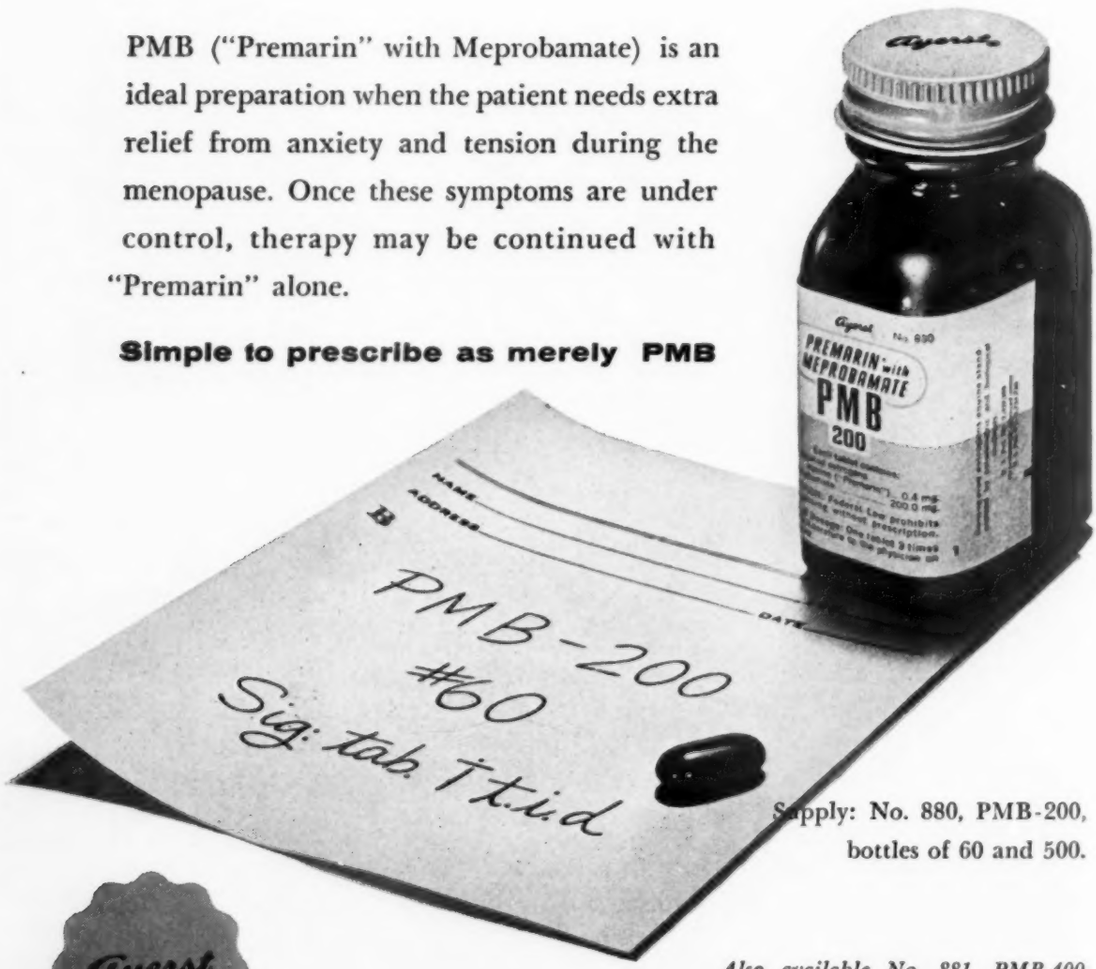
PMB-200

"Premarin" with Meprobamate

Each tablet contains 0.4 mg. "Premarin," 200 mg. meprobamate.

PMB ("Premarin" with Meprobamate) is an ideal preparation when the patient needs extra relief from anxiety and tension during the menopause. Once these symptoms are under control, therapy may be continued with "Premarin" alone.

Simple to prescribe as merely PMB



Supply: No. 880, PMB-200, bottles of 60 and 500.

Also available No. 881, PMB-400 ("Premarin" 0.4 mg. with meprobamate 400 mg.), bottles of 60 and 500.

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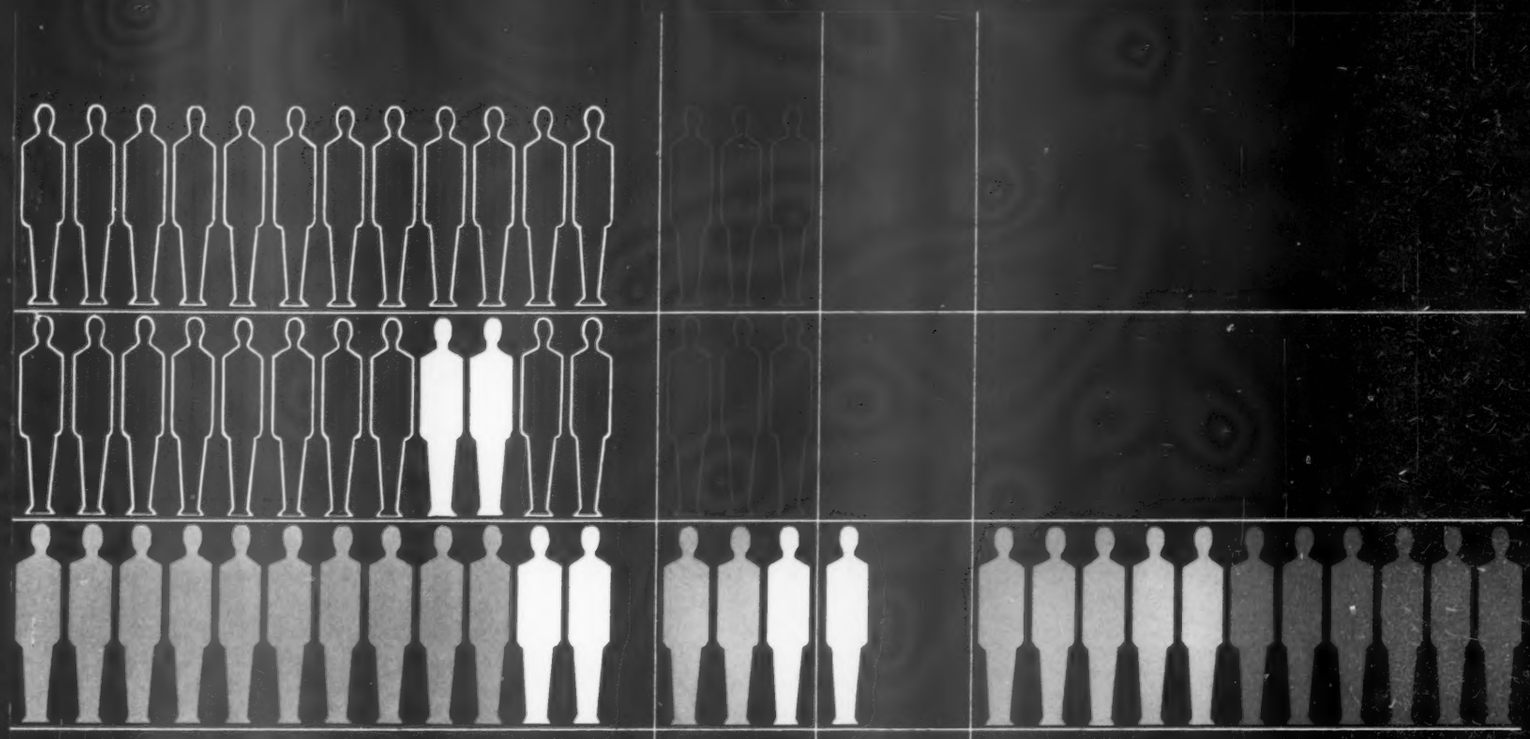

TO TREAT MORE PATIENTS MORE EFFECTIVELY

A new order of magnitude
in therapeutic
effectiveness!

Decadron

DEXAMETHASONE

THE CROWNING ACHIEVEMENT OF THE FIRST CORTICOSTEROID DECADE

MSD MERCK SHARP & DOHME

in rheumatoid arthritis

Decadron*

Brings new meaning to corticosteroid potency

Since the introduction of CORTONE® (cortisone) by Merck in 1948, every new corticosteroid claimed higher potency and lower dosage. These claims were valid so far as dosage was concerned—milligram for milligram, each new steroid was more potent than the preceding one.

But—until DECADRON—higher potency did not mean higher effectiveness. The anti-inflammatory action of other steroids was not superior to that of original cortisone. The dosage was lower, the incidence and character of reactions were different, but the therapeutic effects were about the same.

With DECADRON, however, for the first time in corticosteroid history, increased potency means increased therapeutic usefulness.

Enhances anti-inflammatory effect

"Initial studies on dexamethasone have revealed that it possesses greater antirheumatic potency than any steroid thus far synthesized," report Bunim and coworkers.[1] Boland[2] comments on "strikingly augmented anti-inflammatory potency without corresponding disturbance of electrolyte metabolism." Clinical experiences of Spies and coworkers,[3] who treated various severe allergic and rheumatic conditions with DECADRON, indicate "the striking potency of this material. . . . The acute symptoms, irrespective of diagnosis, faded strikingly."

Often increases the degree of improvement

Of 55 patients with chronic rheumatoid arthritis, treated by Boland and Headley,[4] 17 had achieved an adequate degree of control on prednisolone. When transferred to DECADRON, 12 of these patients retained satisfactory control, but "the degree of improvement advanced from marked to very marked in 5 patients."

Attains satisfactory control in many recalcitrant cases

Even more impressive were the results achieved with DECADRON in severe cases, resistant to treatment with other corticosteroids. Boland and Headley[4] transferred 27 such patients from prednisolone to DECADRON, "At the end of 3 to 5 months improvement was elevated to adequate levels in more than one-third of the group." Bunim and coworkers[5] gave DECADRON to 18 patients with rheumatoid arthritis "who had failed to respond satisfactorily to antirheumatic agents in current use, including the older steroids given in conventional doses. Twelve of the 18 patients showed marked or moderate objective and subjective improvement, four improved slightly and two failed to improve."

Reaches a new height of therapeutic ratio

Significantly, this increase in therapeutic effectiveness was not accompanied by any increase in side effects. "The total number of adverse reactions did not change materially," comment Boland and Headley.[4] They noted none of the "peculiar reactions which sometimes occur with triamcinolone," . . . no clinical evidences of salt and water retention, no elevation in blood pressure. "Nervous reactions have been minimal . . . digestive symptoms have been few." Three patients with diabetes mellitus showed no aggravation of their condition. In the group treated by Bunim and coworkers[5] there was no incidence of edema, peptic ulcers, fractures, mental changes, hypertension, glycosuria, or hyperglycemia.

This increase of therapeutic effectiveness, associated with a low incidence of side effects, means that DECADRON possesses an unsurpassed therapeutic ratio.

References:

1. Bunim, J.J., et al.: Arthr. & Rheum. *1*:313 (Aug.) 1958. 2. Boland, E.W.: California Med. *88*:417 (June) 1958. 3. Spies, T.D., et al.: South. Med. J. *51*:1066 (Aug.) 1958. 4. Boland, E.W., and Headley, N.E.: Paper read before the Am. Rheum Assoc., June 21, 1958, San Francisco, Calif. 5. Bunim, J.J., et al.: Paper read before the Am. Rheum. Assoc., June 21, 1958, San Francisco, Calif.

Decadron*

DEXAMETHASONE

to treat more patients more effectively

Extensive and thorough clinical trials show that

- DECADRON is the most potent of all available oral anti-inflammatory agents
- DECADRON does not usually produce sodium retention, potassium depletion, hypertension or edema
- DECADRON has little or no hyperglycemic activity—in therapeutic doses
- DECADRON has not caused any new or unusual reactions
- DECADRON has produced neither euphoria nor depression, but a healthy sense of well-being

Indications: All allergic and inflammatory disorders amenable to corticosteroid therapy.

Contraindications: Absolute contraindications include active, questionably healed, or suspected tuberculosis, and herpes simplex of the eye. Relative contraindications, in which DECADRON must be administered with caution, are acute or chronic infections, peptic ulcer, osteoporosis, fresh intestinal anastomoses, diverticulitis, thrombophlebitis, pregnancy and psychotic tendencies.

Dosage and Administration: With proper adjustment of dosage, treatment may ordinarily be changed over to DECADRON from any other corticosteroid on the basis of the following milligram equivalence:

One 0.75 mg. tablet of Decadron (dexamethasone) replaces:

↓ One 4 mg.	↓ One 5 mg.	↓ One 20 mg.	↓ One 25 mg.
tablet of methylprednisolone or triamcinolone	tablet of prednisolone or prednisone	tablet of hydrocortisone	tablet of cortisone

Supplied: As 0.75 mg. scored pentagon-shaped tablets; also as 0.5 mg. tablets to provide maximal individualized flexibility of dosage management.

Detailed literature is available to physicians on request.

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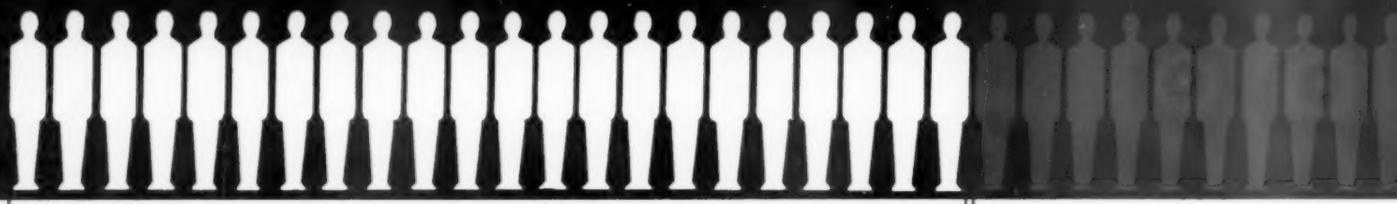
increased therapeutic effectiveness

The increased therapeutic effectiveness of DECADRON can be seen from the following summary of the results reported by Boland and Headley[4] and by Bunim and coworkers.[5] Boland and Headley[4] transferred 44 patients from prednisolone to DECADRON; Bunim and coworkers[5]—18 patients. The status of this total of 62 patients on prednisteroids was as follows:

On Prednisteroids



POOR RESULTS **22**

SLIGHT OR IN

On DECADRON When transferred to **DECADRON**, these patients showed:

POOR RESULTS **4**

SLIGHT OR INADEQUATE IMPROVEMENT **19**

AD

Thus, the incidence of adequate control on DECADRON almost doubled—from 19% on prednisolone, to 37% on DECADRON. The incidence of marked or very marked improvement was more than triple—from 8% on prednisolone, to 26% on DECADRON.

ss of Decadron in rheumatoid arthritis

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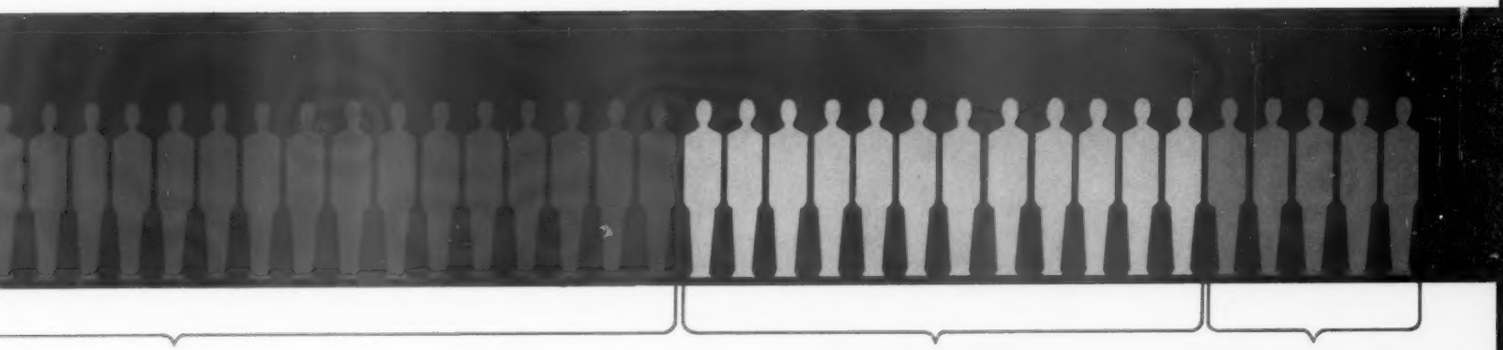
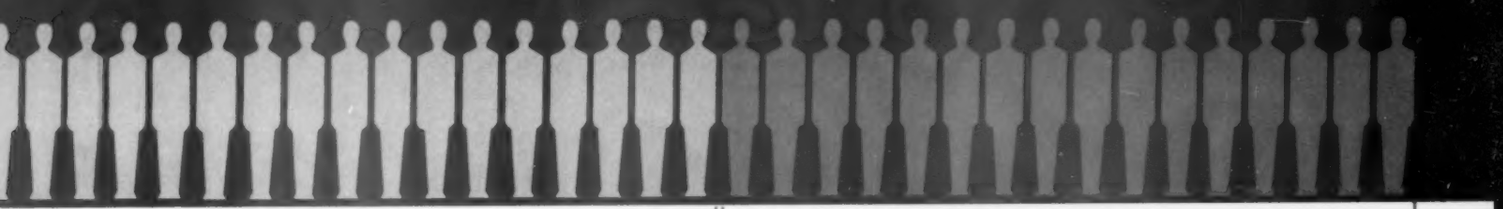


T OR INADEQUATE IMPROVEMENT 23

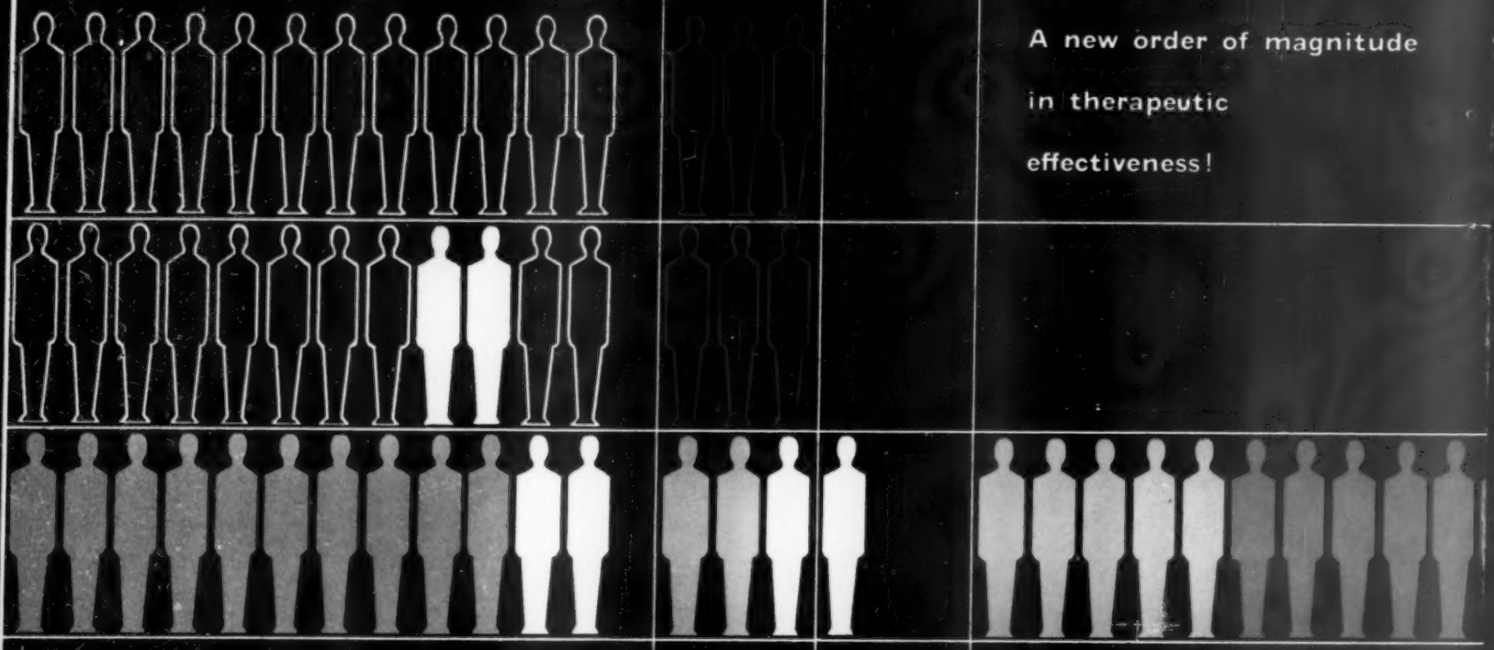
ADEQUATE CONTROL 12

MARKED OR VERY
MARKED IMPROVEMENT 5



ADEQUATE CONTROL 23

MARKED OR VERY MARKED IMPROVEMENT 16



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TO TREAT MORE PATIENTS MORE EFFECTIVELY

Enhanced Anti-inflammatory Potency
Lowest Effective Corticosteroid Dosage
Absence of Any New or Unusual Reactions
Low Incidence of Side Effects
Increase in Therapeutic Usefulness
Increase in Therapeutic Range

Decadron*

DEXAMETHASONE

THE CROWNING ACHIEVEMENT OF THE FIRST CORTICOSTEROID DECADE

MSD MERCK SHARP & DOHME

THE HEART DISEASE PATIENT NEEDS RELIEF FROM EMOTIONAL STRESS

ANXIETY INTENSIFIES the physical disorder in heart disease. "The prognosis depends largely on the ability of the physician to control the anxiety factor, as well as the somatic disease."

(Friedlander, H. S.: The role of ataraxics in cardiology. Am. J. Cardiol. 1:395, March 1958.)

TRANQUILIZATION WITH MILTOWN enhances recovery from acute cardiac episodes and makes patients more amenable to necessary limitations of activities.

(Waldman, S. and Pelner, L.: Management of anxiety associated with heart disease. Am. Pract. & Digest Treat. 8:1075, July 1957.)

Miltown®

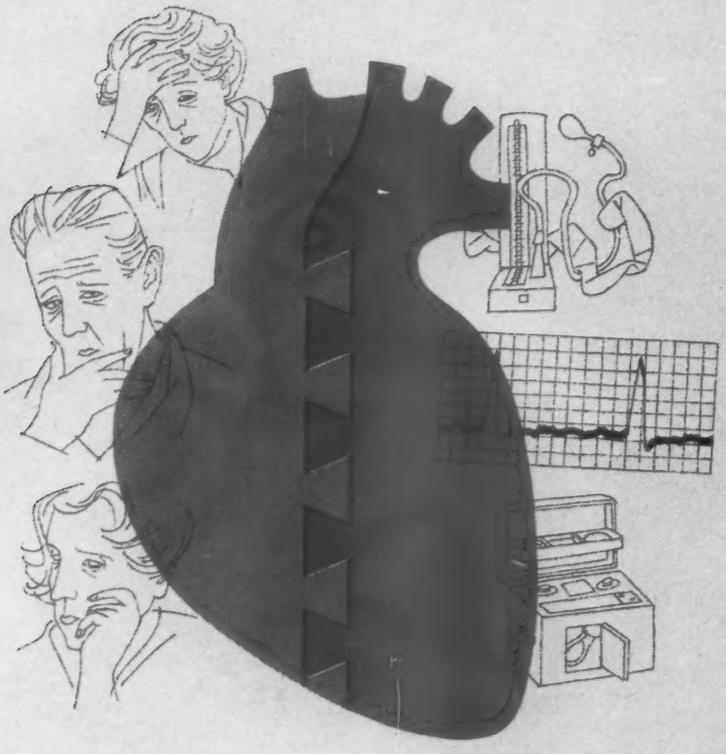
meprobamate (Wallace)

Available in 400 mg. scored and 200 mg. sugar-coated tablets. Also available as MEPROSPAN* (200 mg. meprobamate *continuous release* capsules). In combination with a nitrate, for angina pectoris: MILTRATE*—(Miltown 200 mg. + PETN 10 mg.). *TRADE-MARK CM-7726

Miltown causes no adverse effects on heart rate, blood pressure, respiration or other autonomic functions.

WALLACE LABORATORIES, *New Brunswick, N. J.*

for prevention
of
angina attacks
more effective
than vasodilators alone



Miltrate*

MILTOWN® + PETN

The long-acting nitrate, PETN, helps maintain normal myocardial metabolism while Miltown relieves fear, anxiety and tension. As a result, Miltrate controls both physical and emotional causes of angina attacks.

Miltrate increases exercise tolerance

- reduces nitroglycerin dependence
- is notably safe for prolonged use
- provides convenient one-tablet dosage

Supplied: Bottles of 50 tablets.

Each tablet contains: 200 mg. Miltown + 10 mg. pentaerythritol tetranitrate.

Usual dosage: 1 or 2 tablets q.i.d. *before meals* and at bedtime. Dosage should be individualized.

References:

1. Shapiro, S.: Observations on the use of meprobamate in cardiovascular disorders. Angiology 8:504, Dec. 1957.
2. Friedlander, H. S.: The role of ataraxics in cardiology. Am. J. Cardiol. 1:395, March 1958.
3. Eskwith, I. S.: The holistic approach to angina pectoris. Am. Heart J. 55:621, April 1958.

WALLACE LABORATORIES, *New Brunswick, N. J.*

*TRADE-MARK

CML-8237-19

help reduce
the pressures
IN your
patients

help reduce
the pressures
ON your
patients

for total management of your hypertensive patients rely upon

RAUDIXIN

Squibb Whole Root Rauwolfia Serpentina

Raudixin provides gradual, sustained lowering of blood pressure in hypertensive patients, as well as a mild bradycardia. Hence, the work load of the heart is reduced.

"... often preferred to reserpine in private practice because of the additional activity of the whole root."

Cerrin, K. M.: Am. Pract. & Dig. Treatment 8:721 (May) 1957.

Tranquilizing Raudixin helps relax the anxious hypertensive patient so that he is better able to cope with external pressures without being overwhelmed by them. By reducing these anxieties and tensions, Raudixin helps break the mental tension —hypertension cycle.

Dosage: **Two 100 mg. tablets once daily; may be adjusted within range of 50 to 300 mg.** ***Supply:*** **50 and 100 mg. tablets. Bottles of 100, 1000 and 5000.**

SQUIBB *Squibb Quality—the Priceless Ingredient*

RAUDIXIN® IS A SQUIBB TRADEMARK

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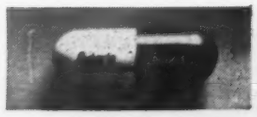
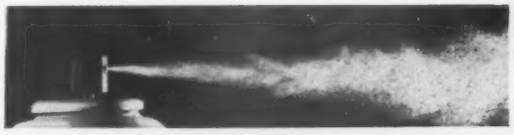
every antibiotic 

need  ...

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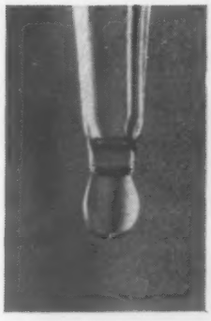
ACHROMYCIN Tetracycline

ACHROMYCIN V Tetracycline with Citric Acid Lederle

the most widely used  and useful... antibiotic

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DOSAGE: *Adults and Children over 8 years*—
1 or 2 tablets, three times daily.
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Each tablet contains: 'Sudafed'® brand Pseudoephedrine Hydrochloride, 30 mg.; 'Perazil'® brand Chlorcyclizine Hydrochloride, 25 mg. Bottles of 100, sugar-coated.

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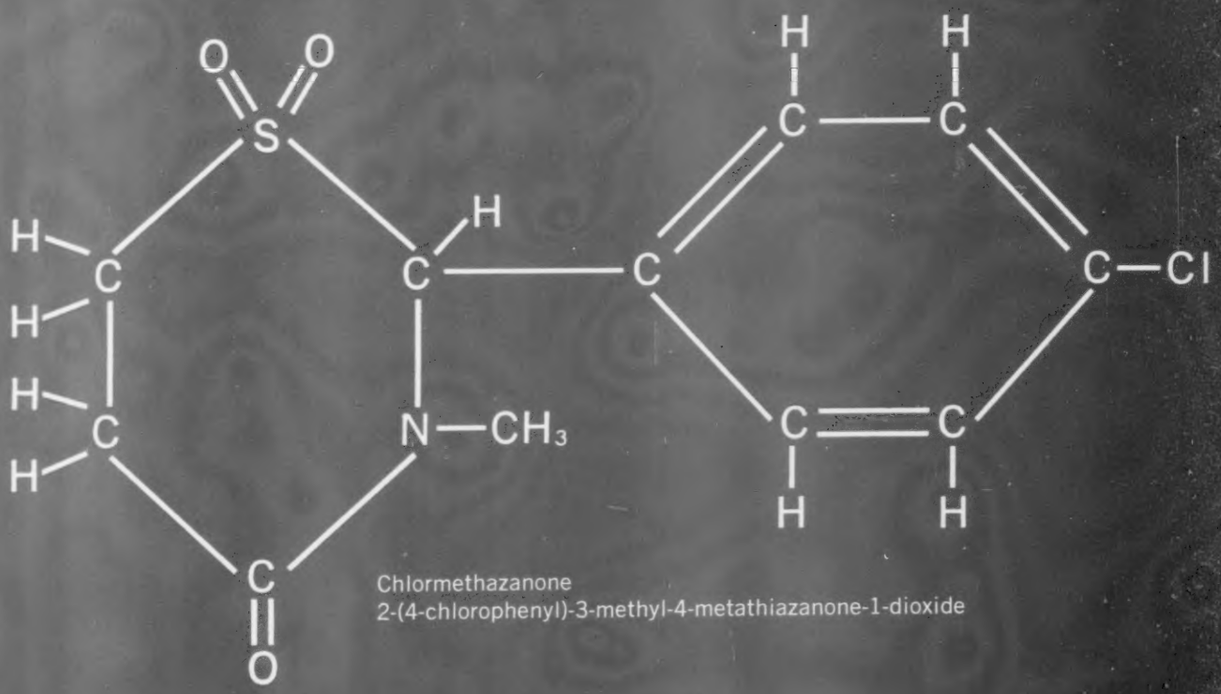
Trancopal®

BRAND OF CHLORMETHAZANONE

a completely new major chemical contribution to therapeutics



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unrelated chemically to any other drug in current use

designed to be equally effective as both

a MUSCLE RELAXANT

a TRANQUILIZER

Trancopal

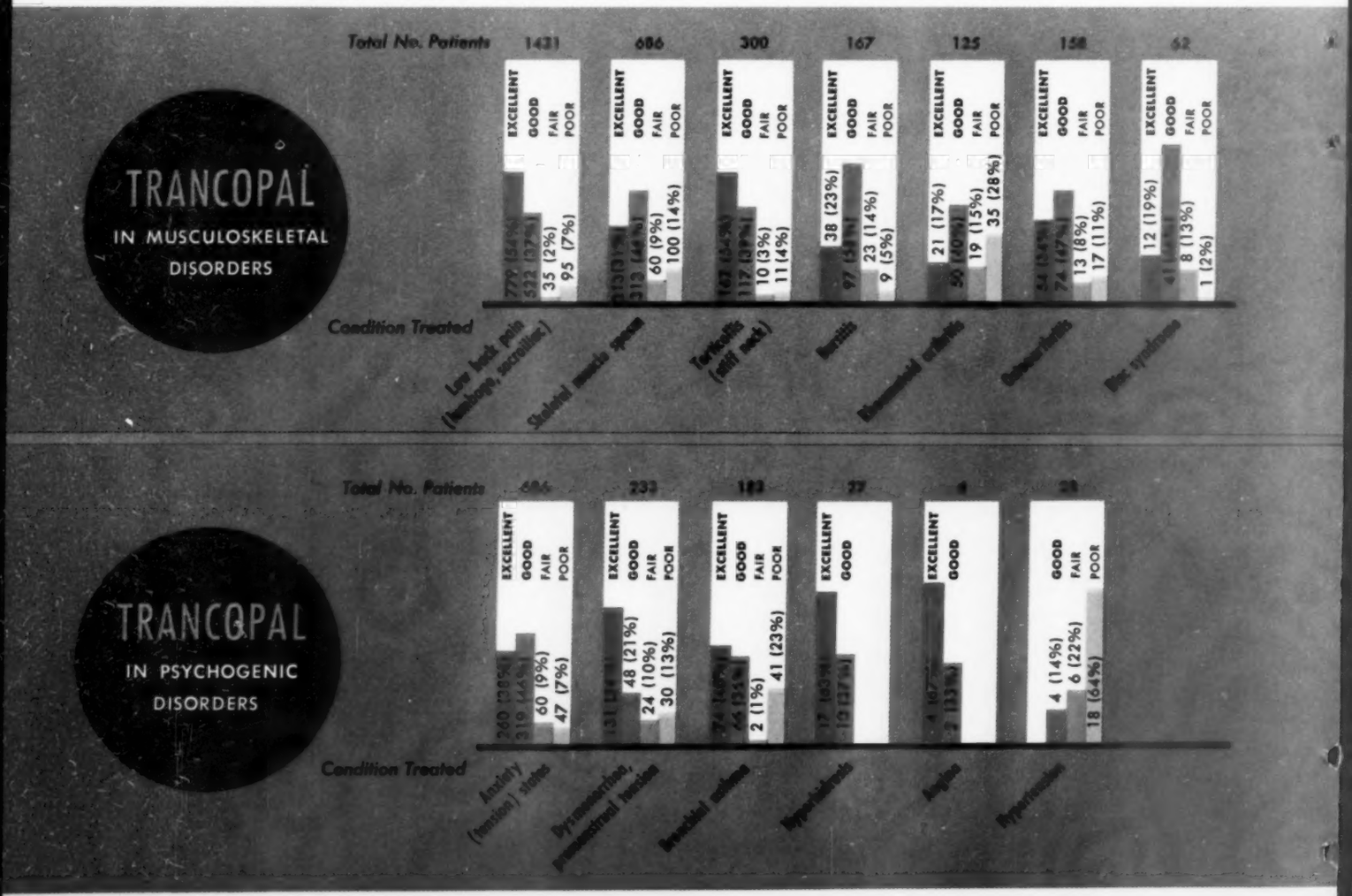
*the first true "TRANQUILAXANT"**

offering new freedom for your patients... from muscle spasm, from tension and anxiety, from side effects

* **tran-qui-lax-ant** (tran'kwi-lak'sant) [< L. tranquillus, quiet; L. laxare, to loosen, as the muscles]

EXCEEDS OLDER DRUGS UP TO 4 TIMES IN PERCENTAGE OF CLINICAL EFFICACY (Lichtman)

The results of clinical studies of over 4000 patients by 105 physicians demonstrate that TRANCOPAL often is effective when other drugs have failed. From these studies it is clear that TRANCOPAL probably can provide more help for a greater number of tense, spastic, and/or emotionally upset patients than any other chemotherapeutic agent in current use.



TRANCOPAL... the first true "tranquilaxant"

Both a muscle relaxant and a calmative agent.

In musculoskeletal disorders, 91 per cent effective.

In anxiety and tension states, 93 per cent effective.

Lower incidence of side effects than with zoxazolamine, methocarbamol or meprobamate.

No known contraindications. Blood pressure, pulse rate, respiration and digestive processes unaffected by therapeutic dosage. No effects on hematopoietic system or liver and kidney function.

Low toxicity. In animals, even less toxic than aspirin.

No gastric irritation. Can be taken before meals.

No clouding of consciousness, no euphoria or depression.

No perceptible soporific effect, even in high dosage.

CLINICAL RESULTS IN 4092 PATIENTS**

MUSCULOSKELETAL CONDITIONS
2929 Patients

+

PSYCHOGENIC CONDITIONS
1163 Patients

=

TOTAL 4092 Patients

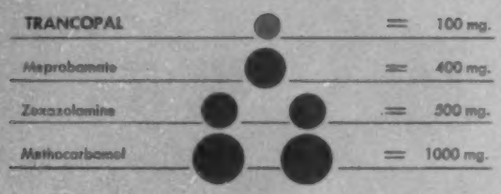
MAJOR IMPROVEMENT
84%

**Cooperative Study, Department of Medical Research, Winthrop Laboratories.

Compare Trancopal with 3 widely used central relaxants

FOR ACTIVITY

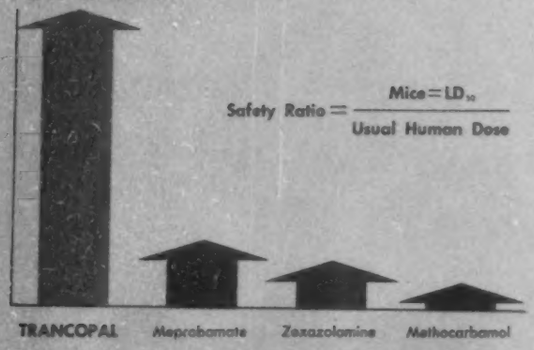
Single Dose



Daily Dose
Same as above, t.i.d.

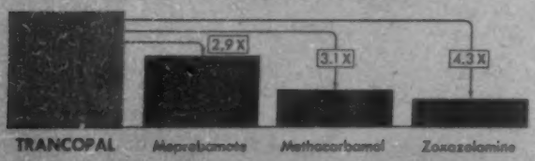
Considering the usual human dose, Trancopal, the first true "tranquilaxant," is four to ten times as potent per milligram.

FOR SAFETY



Comparative pharmacologic tests showed that Trancopal is up to thirteen times as safe, or up to thirteen times less toxic. The measure of safety was the LD_{50} in mice/usual human dose.

FOR CLINICAL EFFECTIVENESS



A clinical comparison in low back pain, torticollis, bursitis and anxiety states showed that Trancopal is up to four times as effective. Each of 40 patients received all four drugs in random rotation for several days. While each of the four drugs gave some relief, only the one providing the most effective relief was recorded.

INDICATIONS

Musculoskeletal
- Low back pain (lumbago)
- Neck pain (torticollis)
- Bursitis
- Rheumatoid arthritis
- Osteoarthritis
- Disc syndrome
- Fibrositis
- Joint disorders (ankle sprain, tennis elbow, etc.)
- Myositis
- Postoperative myalgias

Psychogenic
- Anxiety and tension states
- Dysmenorrhea
- Premenstrual tension
- Asthma
- Emphysema
- Angina

Neurologic
- Muscle spasm in paralysis agitans, multiple sclerosis, hemiplegia, poliomyelitis

TRANCOPAL thoroughly evaluated clinically

"In the treatment of conditions associated with skeletal muscle spasm there was a high percentage of satisfactory results (excellent, good or fair) in 310 patients (94%) out of 331 treated. . . . In 120 patients with simple anxiety or tension states results were satisfactory in 114 (95%). Dosage of chlormethazanone in all cases was 100 mg. t.i.d. As well as relieving the anxiety or tension state, chlormethazanone also allowed these patients to resume their usual occupations."

(Lichtman)

"The effect of this preparation in these cases [skeletal muscle spasm] was excellent and prompt . . ."

Trancopal ". . . was effective in relieving the symptoms of anxiety . . . [with a] profile of pharmacologic actions similar to meprobamate . . ."

(Mullin and Epifano)

"We have just started using it [Trancopal] for relaxing spastic musculature and are very much encouraged."

(Baker)

Trancopal

the first true "TRANQUILAXANT"

Dosage: One Caplet (100 mg.) orally three or four times daily. Relief of symptoms occurs in fifteen to thirty minutes and lasts from four to six hours.

Supplied: Trancopal Caplets® (scored) 100 mg., bottles of 100.

Winthrop Laboratories • New York 18, N. Y.

• Baker, A. B.: Modern Med. 26:140, April 15, 1958. • Cohen, A. I.: In preparation. • Cooperative Study, Department of Medical Research, Winthrop Laboratories. • Gesler, R. M., and Coulston, F.: Toxicol. & Appl. Pharmacol. To be published. • Gesler, R. M., and Surrey, A. R.: J. Pharmacol. & Exper. Therap. 122:24A, Jan., 1958. • Gesler, R. M., and Surrey, A. R.: J. Pharmacol. & Exper. Therap. 122:517, April, 1958. • Lichtman, A. L.: Kentucky Acad. Gen. Pract. J. 4:28, Oct., 1958. • Mullin, W. G., and Epifano, Leonard: To be published. • Surrey, A. R.; Webb, W. G., and Gesler, R. M.: J. Am. Chem. Soc. 80:3469, July 5, 1958.

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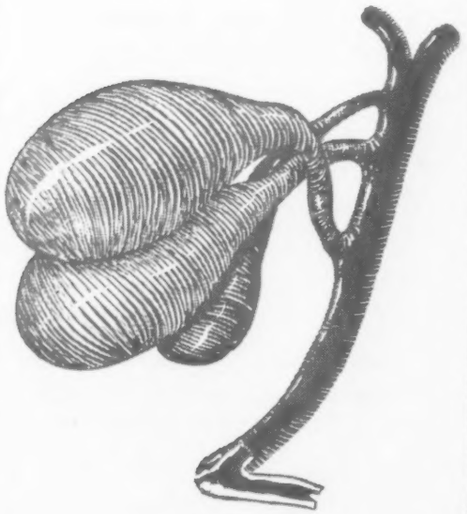
One hundred and twenty-two cases of *vesica fellea divisa* (bilobed gallbladder) and *vesica fellea duplex* (double gallbladder with 2 cystic ducts) are reported in the literature. A unique case of *vesica fellea triplex* has recently been described.

Source: Skilboe, B.: Am. J. Clin. Path. *30*:252, 1958.



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free-flowing bile
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(1) Beckman, H.: Drugs: Their Nature, Action and Use, Philadelphia, W. B. Saunders Company, 1958, p. 425.
(2) Biliary Tract Diseases, M. Times *85*:1081, 1957.

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Exactly how does new Halodrin* restore the "premenopausal prime" in postmenopausal women?

Webster defines "prime" as the period of greatest health, strength, and beauty. In a woman, these are the childbearing years between puberty and menopause—the years when her hormone production is highest.

The inevitable reduction in this hormone production as she enters the menopause often results in physical discomfort in the form of hot flushes, nervousness, insomnia, or a multiplicity of other symptoms with which you are familiar. Superimposed on this physical picture is the psychic trauma brought on by this unavoidable evidence of aging. The thing that brings her to a physician is simply that she "feels bad."

You can't make her 35 again—but the odds are good that you can make her feel like it! The secret is a combination of reassurance and hormones. The exact form and amount of the former defy objective analysis, but the latter can now be provided with scientific precision. Reduced to essentials, here is the explanation of exactly how hormones—in the form of Upjohn's new Halodrin—restore the "premenopausal prime."

The normal premenopausal woman excretes estrogens in the urine in the form of estradiol, estrone, and estriol, in an approximate 28-day average ratio of 39:15:46. Starting with this urinary excretion of estrogens, it is possible to calculate backwards and estimate the amount of estradiol that must have been secreted endogenously in order to produce these urinary levels. This is possible because the proportion of estrogens which appears in the urine following parenteral administration has been established in castrated women.

On this basis, the average endogenous output of estrogens is about 160 micrograms per day during a menstrual cycle, and 80 micrograms per day in postmenopausal women (see chart opposite). Therefore, the restoration of the "premenopausal prime" in the postmenopausal woman requires the replacement of approximately the equivalent of the 80 micrograms of estradiol per day that she no longer secretes endogenously.

Oral ethinyl estradiol is about 2 to 2½ times as potent as parenteral estradiol. Therefore, **the replacement of 80 micrograms of endogenous estradiol production per day is accomplished by the oral administration of 32 to 40 micrograms of ethinyl estradiol per day.**

Each Halodrin tablet contains 20 micrograms of ethinyl estradiol, which means that the recommended dosage of 2 tablets per day provides 40 micrograms of ethinyl estradiol. This offsets the loss of 80 micrograms of endogenous estradiol production in the menopausal woman; i.e., restores the "premenopausal prime."

Each Halodrin tablet also contains 1 mg. of Upjohn-developed Halotestin* (fluoxymesterone)—the most potent oral androgen known. The primary purpose is to "buffer" the ethinyl estradiol just enough to prevent breakthrough bleeding, which is obviously undesirable in the menopause. It also exerts other beneficial hormonal effects, one of which, in common with ethinyl estradiol, is a powerful anabolic action so desirable in patients of advanced years.

Upjohn

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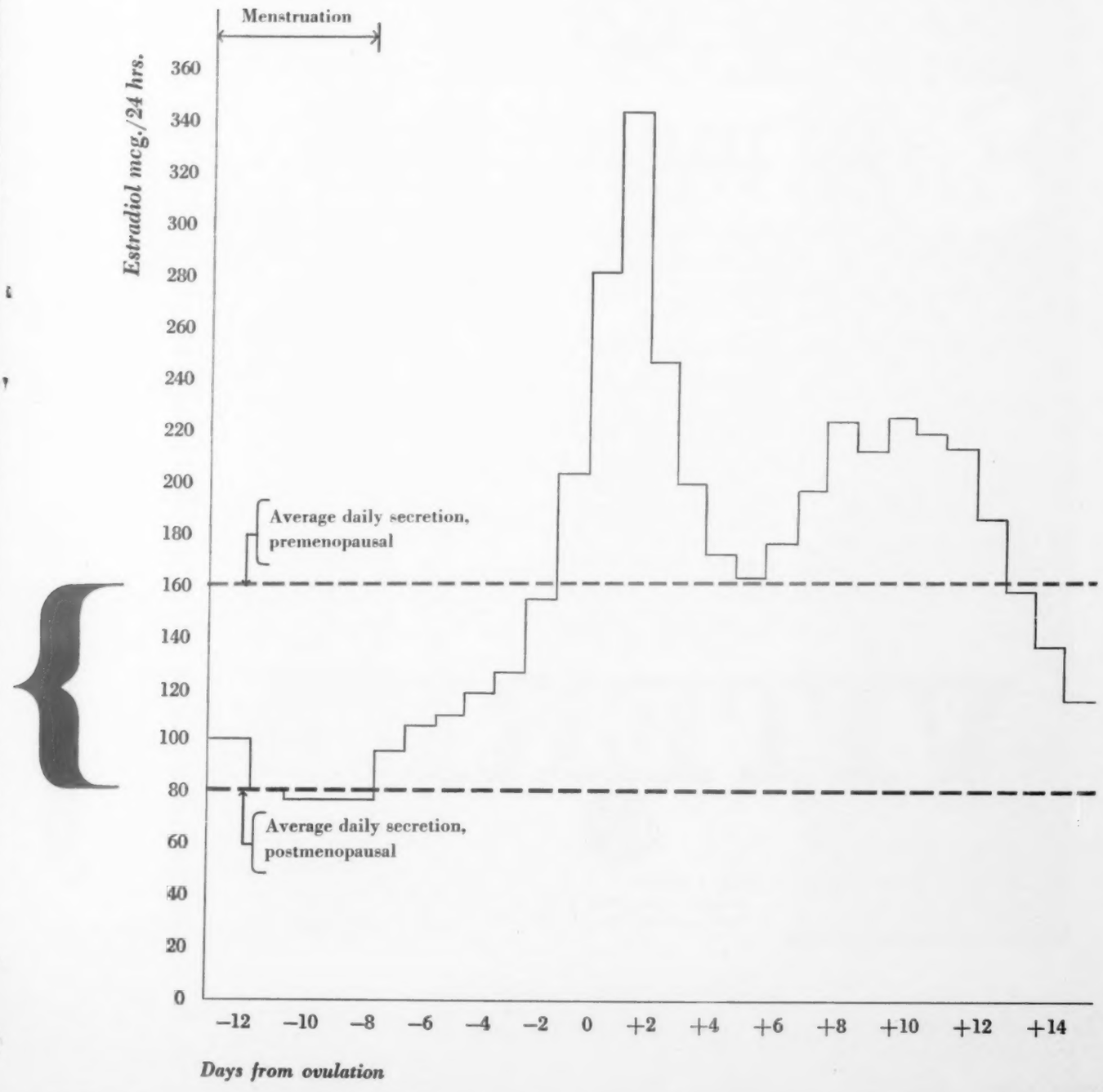
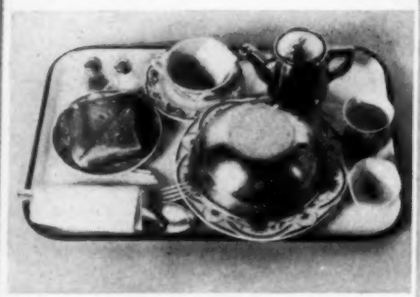
Endogenous estrogen secretion (mcg/24 hours)
(calculated from average 24-hour urinary excretion of estradiol, estrone, and estriol)





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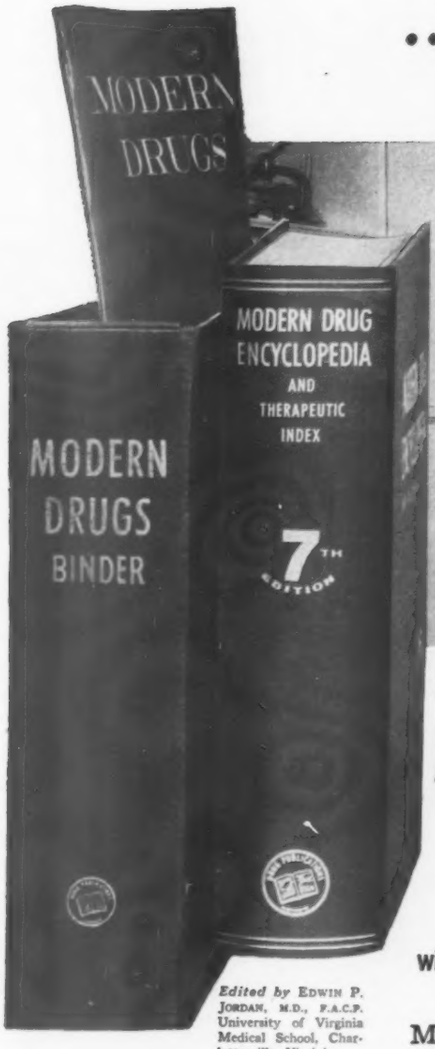

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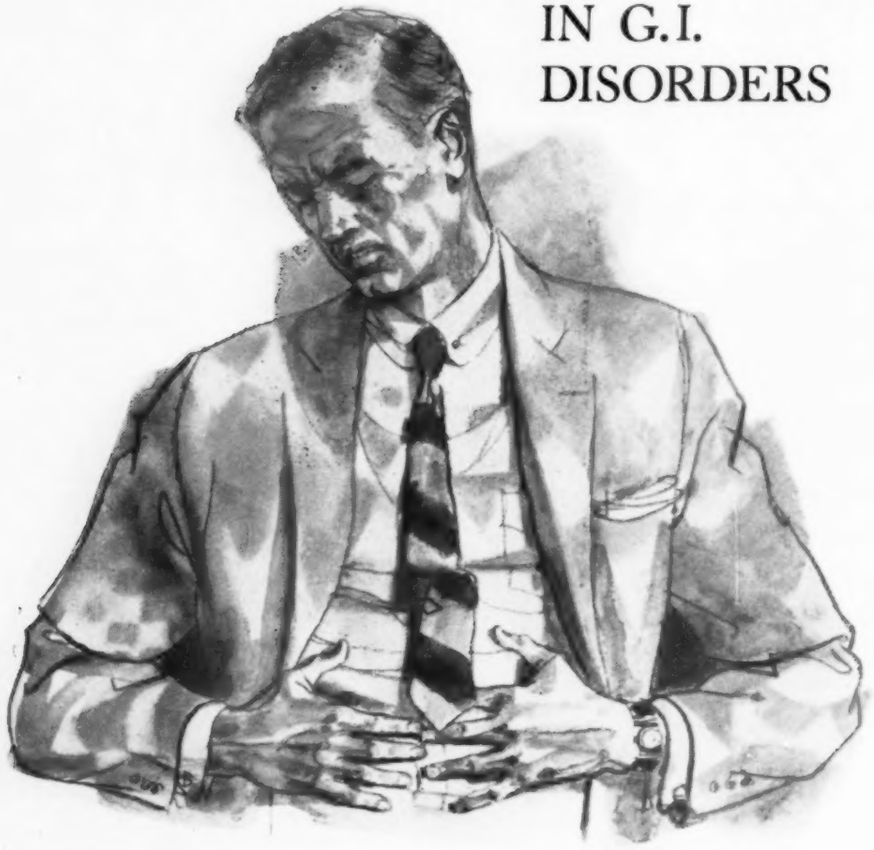

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1. Phillips, R. E.: Am. Pract. & Digest Treat. 7:1573, Oct. 1956. 2. Selling, L. S.: J.A.M.A. 157:1594, April 30, 1955. 3. Altschul, A. and Billow, B.: New York J. Med. 57:2361, July 15, 1957. 4. Ross, S. T.: Postgrad. Med. 23:24, Jan. 1958. 5. Tacket, H. S.: Am. Pract. & Digest Treat. 8:597, April 1957. 6. Bodi, T., Wirts, C. W., Jr. and Menduke, H.: Am. J. Gastroenterol. 29:643, June 1958.

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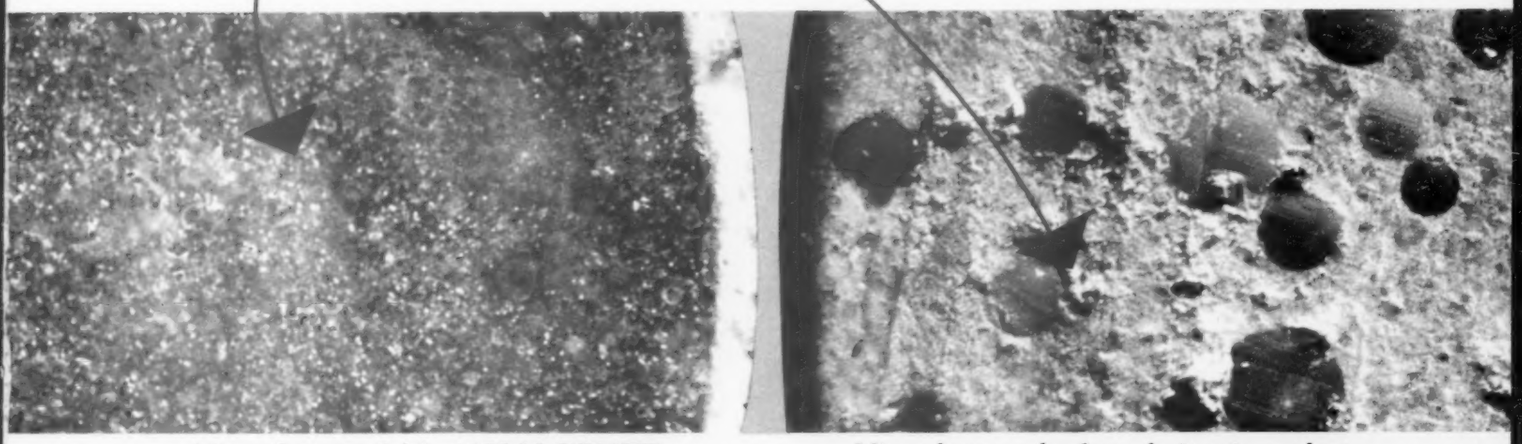

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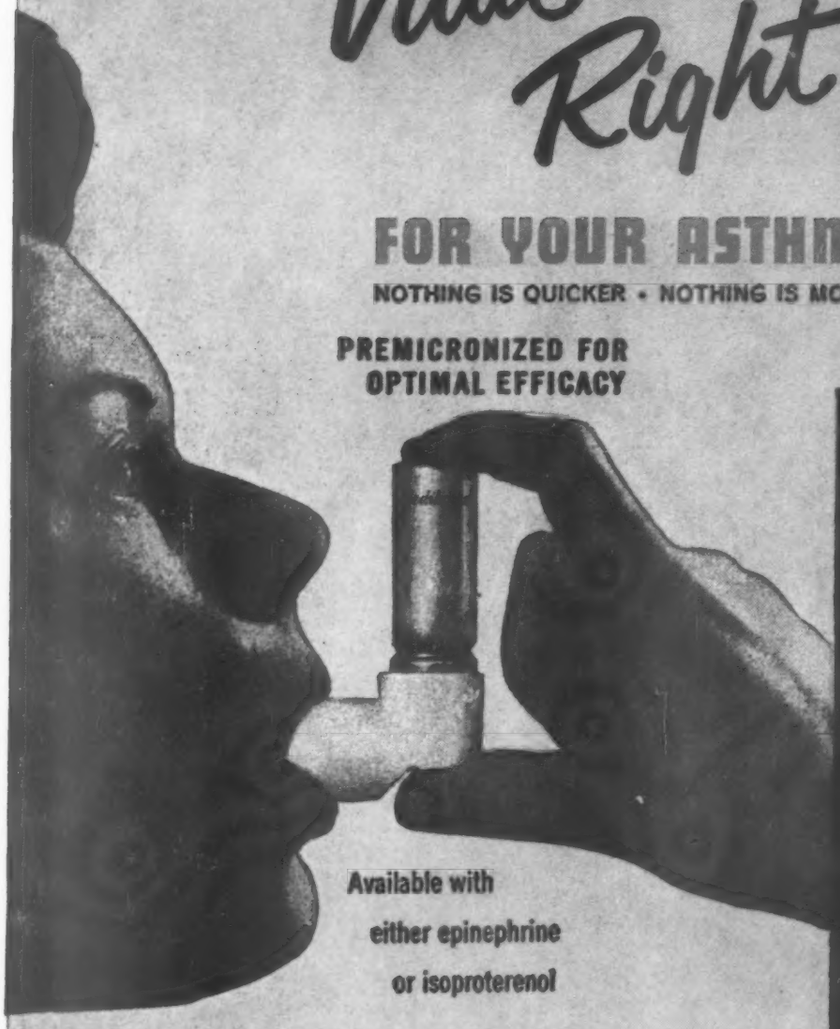

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